University of Alberta

Mapping the protective epitope of an anti-Candida albicans monoclonal antibody using synthetic di- and trisaccharide analogues of (1-2)-β-D-mannopyranoside

by

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requirements for the degree of *Doctor of Philosophy*

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Abstract

The specific recognition of glycans by proteins is essential for many biological processes. Carbohydrate antigens on the surface of pathological agents are bound by immunoglobulins and in some instances this interaction provides a protective mechanism for the host. Deliberately targeting these structures to elicit an immune response forms the therapeutic basis for development of synthetic glycoconjugate vaccines. Knowledge of the recognition elements of the protective epitope can provide important insights into the ideal minimum sized hapten that can be employed. Previously, a monoclonal antibody, C3.1, protective in mice against *Candida albicans* has been identified and shown from inhibition studies to bind a trisaccharide of (1→2)-β-D-mannan with significantly higher affinity than larger oligomers. The aim of the present study is to provide a detailed description of the size and topology of the protective epitope contained in the (1→2)-β-D-mannopyranoside recognized by mAb C3.1.

Sixteen analogues of both a disaccharide **1** and trisaccharide **2** of (1→2)-β-D-mannopyranoside have been synthesised. The analogues include structures with hydroxyl groups independently replaced with hydrogen and *O*-methyl functionalities. Epimers of the C-2′ and C-2″ positions were also included. Inclusion of the analogues in a competitive ELISA indicated that compounds modified at C-3, C-4, C-4′ or C-6′ showed significant reduction in inhibitor activity compared to the reference disaccharide **1** suggesting that these hydroxyl groups are important for recognition by mAb C3.1. Modifications to the other hydroxyl groups were tolerated. From a global minimum model of the disaccharide it appears that the hydroxyl groups, essential for ligand binding, form a continuous contact surface located along one edge of the helical epitope.

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Table of Contents

Chapter	Title	Page
Chapter 1	**Introduction: Recognition of carbohydrates by antibody binding sites**	**1**
1.1	General introduction	1
1.2	Recognition of carbohydrate antigens by antibody binding sites	6
1.2.1	Carbohydrate antigens	6
1.2.2	The immune response to carbohydrate antigens	8
1.2.3	The antibody molecule	13
1.2.4	Production and purification of monoclonal antibodies	17
1.3	The thermodynamics of carbohydrate-protein interactions	18
1.3.1	Intermolecular forces and the association constant	18
1.3.2	Thermodynamic parameters of binding	19
1.3.3	Quantification of carbohydrate-protein interactions	21
1.3.4	Chemical mapping of carbohydrate epitopes	22
1.4	*Candida albicans*	28
1.4.1	Background	28
1.4.2	The phosphomannan complex of *Candida albicans*	29
1.4.3	Monoclonal antibodies specific for the PMC	31
1.5	Scope of project	32
Chapter 2	**Synthesis of methyl (1→2)-β-D-mannopyranoside analogues**	**35**
2.1	Introduction: Synthetic aspects of carbohydrate chemistry	35
2.1.1	Glycoside formation	36

Chapter	Title	Page
2.1.2	Factors controlling anomeric stereochemistry during glycoside formation	39
2.1.2.1	Anomeric effect	39
2.1.2.2	Neighbouring group participation	40
2.1.3	Literature methods for the synthesis of β-D-mannopyranosides	43
2.1.3.1	Activation of mannosyl halides with insoluble promoters	43
2.1.3.2	Formation of β-D-mannopyranosides via inversion at C-2	44
2.1.3.3	The ulosyl bromide method	47
2.1.3.4	Intramolecular aglycone delivery	48
2.1.3.5	Displacement with inversion of α-mannosyl triflate donors	50
2.2	Synthesis of methyl β-D-mannopyranosyl-(1→2)-β-D-mannopyranoside and deoxy and *O*-methyl analogues	52
2.2.1	General considerations and retrosynthetic analysis	52
2.2.2	Synthesis of (1→2)-β-D-mannopyranosyl disaccharide and trisaccharide	59
2.2.3	Synthesis of C-2′ modified disaccharide analogues	62
2.2.4	Synthesis of C-3′ modified disaccharide analogues	64
2.2.5	Synthesis of C-4′ modified disaccharide analogues	66
2.2.6	Synthesis of C-6′ modified disaccharide analogues	70
2.2.7	Synthesis of C-3 modified disaccharide analogues	73
2.2.8	Synthesis of C-4 modified disaccharide analogues	77
2.2.9	Synthesis of C-6 modified disaccharide analogues	80
2.2.10	Synthesis of C-2″ modified disaccharide analogues	83
2.3	Conclusions	84

Chapter	Title	Page
Chapter 3	**Effects of functional group modification on the binding of (1→2)-β-D-mannopyranosides by mAb C3.1**	**85**
3.1	The ELISA protocol	85
3.2	Mapping the protective epitope recognized by mAb C3.1	89
Chapter 4	**Qualitative analysis of trisaccharide binding to mAb C3.1 using saturation transfer difference NMR**	**99**
4.1	General introduction to saturation transfer difference NMR	99
4.2	Features of the STD-NMR experiment	101
4.3	Optimal STD-NMR pulse sequence parameters	103
4.3.1	Placement of the selective on- and off-resonance pulse cascades	104
4.4	STD-NMR analysis of trisaccharide **2** binding to mAb C3.1	110
Chapter 5	**Conclusions and Future Directions**	**114**
Chapter 6	**Experimental**	**118**
	References	**244**

List of Tables

Table	Title	Page
1.1	Inhibition by synthetic (1→2)-β-D-mannopyranoside oligosaccharides of the binding of mAb C3.1 (IgG3) to *C. albicans* PMC.	32
3.1	The effects of hydroxyl group modification on the inhibition by disaccharide **1** of the binding of mAb C3.1 (IgG3) to solid supported (1→2)-β-D-mannan trisaccharide-BSA conjugate.	90

List of Figures

Figure	Title	Page
1.1	The structure of the core pentasaccharide found in *N*-linked glycans.	3
1.2	Glycoconjugate vaccine licensed against *H.* influenzae type b containing the first fully synthetic carbohydrate hapten linked to a carrier protein	7
1.3	B-cell mediated immune response to carbohydrate antigens.	12
1.4	The antibody molecule.	15
1.5	Strategies to chemically map the role of oligosaccharide hydroxyl groups in protein complexes.	25
1.6	The phosphomannan complex.	30
1.7	Synthetic deoxy and *O*-methyl analogues of disaccharide **1** and trisaccharide **2**.	34
3.1	Indirect competitive ELISA protocol used for evaluation of synthetic analogues against mAb C3.1.	88
3.2	Global minimum energy model of a disaccharide portion of a (1→2)-β-D-mannopyranosyl undecasaccharide.	93
3.3	Two available frame-shifted binding modes for a disaccharide occupying the binding site of mAb C3.1.	95
3.4	Global minimum energy models of a trisaccharide portion of a (1→2)-β-D-mannopyranosyl undecasaccharide.	97
3.5	Global minimum energy model of a tetrasaccharide portion of a (1→2)-β-D-mannopyranosyl undecasaccharide.	98
4.1	Illustration of epitope mapping using the technique of saturation transfer difference NMR.	100
4.2	The STD-NMR pulse sequence.	104
4.3	1-D NMR spectra of trisaccharide **2** measured at 600 MHz.	105
4.4	Array of the EBURP1 on-resonance pulse in the aromatic region.	106

Figure	Title	Page
4.5	Array of the EBURP1 on-resonance pulse in the aliphatic region.	107
4.6	Array of the EBURP1 off-resonance pulse in the far-aromatic region.	108
4.7	Control STD-NMR spectrum of trisaccharide **2** measured at 600 MHz.	109
4.8	STD-NMR spectrum of trisaccharide **2** and mAb C3.1 (IgG3) measured at 600 MHz.	112
4.9	The epitope map of trisaccharide **2**.	112

List of Schemes

Scheme	Title	Page
2.1	Synthesis of the different glycoside linkages.	37
2.2	Halide-ion catalyzed glycosidation.	38
2.3	Common glycosyl donor leaving groups.	39
2.4	Rationalizing the anomeric effect.	40
2.5	Neighbouring group participation.	41
2.6	1,2-*cis* α-glycosylation strategy based on neighbouring group participation.	43
2.7	Formation of β-D-mannopyranosides using the insoluble promoter strategy.	44
2.8	Formation of β-D-mannopyranosides *via* the oxidation-reduction method.	45
2.9	Formation of β-D-mannopyranosides *via* displacement.	47
2.10	The ulosyl bromide approach for the formation of β-D-mannopyranosides.	48
2.11	Synthesis of β-D-mannopyranosides by Intramolecular Aglycone Delivery.	50
2.12	Synthesis of β-D-mannopyranoside linkages *via* displacement with inversion of α-mannosyl triflate donors.	52
2.13	Retrosynthesis of disaccharide **1**.	54
2.14	Retrosynthesis of analogues (**6-11**) modified on the non-reducing monosaccharide residue.	57
2.15	Retrosynthesis of analogues (**12-17**) modified on the reducing monosaccharide residue.	58
2.16	Synthesis of β-D-mannopyranosyl-(1→2)-β-D-mannopyranoside **1**.	60

Scheme	Title	Page
2.17	Synthesis of β-D-mannopyranosyl-(1→2)-β-D-mannopyranosyl-(1→2)-β-D-mannopyranoside **2**.	61
2.18	Synthesis of methyl 2′-deoxy analogue **3**.	62
2.19	Synthesis of methyl 2′-*O*-methyl analogue **4**.	63
2.20	Synthesis of methyl 2′-*gluco* analogue **5**.	64
2.21	Synthesis of methyl 3′-deoxy analogue **6**.	65
2.22	Synthesis of methyl 3′-*O*-methyl analogue **7**.	66
2.23	Synthesis of 4′-deoxy thioglucoside donor **45**.	67
2.24	Synthesis of 4′-*O*-methyl thioglucoside donor **47**.	68
2.25	Synthesis of 4′-deoxy **8** and 4′-*O*-methyl **9** analogues.	69
2.26	Synthesis of 6′-deoxy thioglucoside donor **59**.	70
2.27	Synthesis of 6′-*O*-methyl thioglucoside donor **62**.	71
2.28	Synthesis of 6′-deoxy **10** and 6′-*O*-methyl **11** analogues.	72
2.29	Synthesis of methyl 3-deoxy-β-D-mannose acceptor **74**.	74
2.30	Synthesis of methyl 3-*O*-methyl-β-D-mannose acceptor **76**.	75
2.31	Synthesis of 3-deoxy **12** and 3-*O*-methyl **13** analogues.	76
2.32	Synthesis of methyl 4-deoxy-β-D-mannose acceptor **88**.	77
2.33	Synthesis of methyl 4-*O*-methyl-β-D-mannose acceptor **90**.	78
2.34	Synthesis of 4-deoxy **14** and 4-*O*-methyl **15** analogues.	79
2.35	Synthesis of methyl 6-deoxy-β-D-mannose acceptor **101**.	80
2.36	Synthesis of methyl 6-*O*-methyl-β-D-mannose acceptor **106**.	81
2.37	Synthesis of 6-deoxy **16** and 6-*O*-methyl **17** analogues.	82
2.38	Synthesis of 2″-*O*-methyl trisaccharide analogue **18**.	83
2.39	Synthesis of 2″-*gluco* trisaccharide analogue **19**.	84

Abbreviations

Ac	acetyl
AcOH	acetic acid
Ac_2O	acetic anhydride
AIBN	azobisisobutyronitrile
APC	antigen presenting cell
All	allyl
$BF_3{\cdot}OEt_2$	boron trifluoride diethyl etherate
$BH_3{\cdot}THF$	borane tetrahydrofuran complex
Bn	benzyl
BnBr	benzyl bromide
Bz	benzoyl
BzCl	benzoyl chloride
n-Bu_2BOTf	dibutyl boron triflate
n-Bu_3SnH	tributyltin hydride
CSA	camphorsulfonic acid
DBU	1,8-diazabicyclo-[5.4.0]-undec-7-ene
DDQ	dichloro dicyano quinone
DMF	*N,N*-dimethylformamide
DTBMP	di-*tert*-butyl methyl pyridine
ELISA	enzyme linked immunosorbent assay
ESI HRMS	electrospray ionization high resolution mass spectrometry
Et	ethyl
Fab	antigen binding fragment
GlcNAc	*N*-acetylglucosamine, 2-acetamido-2-deoxy-D-glucose
GCOSY	gradient coupling correlated spectroscopy
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum coherence
HRP	horseradish peroxidase
IC_{50}	inhibitor concentration required to give 50% inhibition

Ig	immunoglobulin
t-BuOK	potassium *tert*-butoxide
mAb	monoclonal antibody
Me	methyl
MeCN	acetonitrile
Me_2SO	dimethyl sulfoxide
MHC	major histocompatibility complex
MS	molecular sieves
Ms	methanesulfonyl
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
PBS	phosphate buffered saline
PBST	phosphate buffer saline containing Tween 20
Ph	phenyl
PMB	4-methoxybenzyl
PMC	phosphomannan complex
ppm	parts per million
RT	room temperature
SepPak	solid phase extraction cartridge
S_N2	bimolecular nucleophilic substitution
STD-NMR	saturation transfer difference NMR
TCDI	1,1′-thiocarbonyl diimidazole
TCR	T-cell receptor
TfOH	trifluoromethanesulfonic acid
Tf_2O	trifluoromethanesulfonic anhydride
T_{H1}	T-helper lymphocyte type 1
T_{H2}	T-helper lymphocyte type 2
THF	tetrahydrofuran
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
p-TSA	*p*-toluenesulfonic acid

Chapter One

Introduction: Recognition of carbohydrates by antibody binding sites

1.1 General introduction.

Carbohydrates are recognized as one of the major classes of biomolecules, alongside proteins, nucleic acids and lipids.[1] Complex carbohydrates may exist as linear or branched structures composed of polyhydroxylated monomers. They are ubiquitous and arguably the most abundant of natural products considering the quantities of cellulose, chitin and starch. Organisms from all three domains of the phylogenetic tree, namely the eubacteria, archaeabacteria and eukaryotes, are known to produce carbohydrates.[2,3] However, there is a discontinuous distribution of some structures across evolutionary lines, that is, not all organisms express the same carbohydrate structures.[4] For example, peptidoglycans found in the exoskeleton of bacteria,[5] or chitin found in fungi, arthropods, molluscs and one teleost fish. In nature carbohydrates may exist separately or in covalent association with other classes of molecules; these covalent structures are termed glycoconjugates. It is now well recognized that complex, diverse arrays of glycoconjugates decorate the outer surface of most cells, that proteins in both the cytosol and nucleus are glycosylated[6] and many secreted molecules are in fact glycoconjugates.[7]

A glycoconjugate is a biomolecule formed by the covalent attachment of a mono-, oligo- or polysaccharide to a non-carbohydrate entity (aglycone). The sugar portion of the glycoconjugate is commonly referred to as the glycan. The glycome of eukaryotes includes glycans of glycoproteins, glycolipids (including glycosylphosphatidylinositol

anchors) and proteoglycans. The cell wall of many bacteria contains additional structures including peptidoglycans, lipopolysaccharides and capsular polysaccharides.

Glycoproteins are characterized by the post-translational covalent attachment of a sugar to an amino acid residue of a polypeptide chain.[8,9] In 2002 according to Spiro's report, there were at least 41 different glycoprotein linkages found in nature, which involve glycosylation of 8 different amino acids with 13 different monosaccharides.[2] Since that review, novel glycosidic linkages continue to be discovered.[10,11] Despite the great number of different glycosidic bonds, *N*- and *O*-linked glycans where the amino acid residues involved are asparagine and either serine or threonine still remain the most common.

The most widely distributed linkage of glycoproteins is the covalent attachment of β-GlcNAc to asparagine.[2] The β-GlcNAc-Asn bond has been observed in all three domains of the phylogenetic tree. The glycosylated asparagine residue is part of a consensus peptide sequence Asn-X-Ser/Thr, where X is any amino acid other than proline; though not all of these sequence motifs in a particular protein are glycosylated. *N*-glycans of this type share a common pentasaccharide core region (Figure 1.1). Elaboration of the core forms a wide variety of biologically important *N*-glycoproteins found in nature.

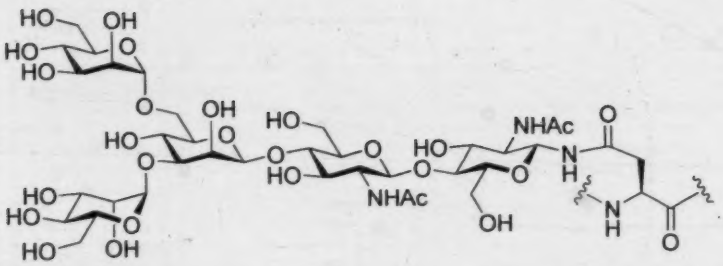


Figure 1.1. *The structure of the core pentasaccharide found in N-linked glycans.*

Attachment of a sugar to the hydroxyl of an amino acid residue in a polypeptide chain forms *O*-linked glycoproteins. In eukaryotes, the α-linkage between *N*-acetylgalactosamine and serine/threonine is characteristic of mucins.[12] The *O*-glycosylation of mucins typically involves smaller glycans often consisting of only a few sugar residues. Post-translational modification of serine/threonine residues of nuclear and cytoplasmic proteins with *O*-linked β-*N*-acetylglucosamine plays an important role in cellular regulation.[13] The occurrence of *O*-linked glycans has been extensively reviewed.[14]

Glycolipids are divided into two structural categories, the glycosphingolipids and glycosyl phosphatidyl inositols (GPI's). The lipid portions of each type are embedded in the membrane bilayer. Glycosphingolipids are characterized by attachment of a monosaccharide, usually glucose or galactose to ceramide. Elaboration of these basic structures gives rise to over 200 distinct glycosphingolipids that have been reported from a wide variety of eukaryotic sources (e.g. GM_1).[15,16] GPI's, are structures that anchor many proteins to the membrane.[17] The GPI anchors share a common structure where phosphatidylinositol is glycosidically linked to the reducing end of glucosamine.

Glycans perform numerous biological functions, which is perhaps not surprising, considering their wide distribution and great structural complexity.[18-20] Though no single

predominant role can be attributed to protein- or lipid-linked glycans, two broad groupings can be defined. The first group include structural and modulatory effects performed by glycans and the second, effects mediated by specific recognition of glycans by other molecules.[21,22] Cell wall polysaccharides of bacteria and fungi (e.g. β-glucans, lipopolysaccharides and capsular polysaccharides) and extracellular matrix of plants (e.g. cellulose, hemicellulose and pectins) provide structural support and protection for the cell. Protein glycosylation also modulates many biophysical properties including: protein folding, solubility, structural dynamics, catalytic activity, stability, and aggregation.[23-28]

Interactions of glycans with a variety of proteins, including antibodies, lectins, and enzymes, are responsible for many biological processes related to both health and disease; including immune response, fertilization, embryonic development, host-microbe interactions and tumor metastasis.[29-36] Glycan interactions mediate diverse cellular activities, such as cell adhesion and migration,[37-39] molecular trafficking and clearance,[40] receptor activation,[41] signal transduction and endocytosis.[42,43] The three-dimensional structures of glycan chains store information that provides a "high density coding system" required for the selective and efficient functioning of these diverse processes.[44] Though many of these individual glycan interactions may be of low-affinity, multivalent binding where two or more ligands interact with a receptor allows for sufficient binding strength and specificity to mediate the communication of biological information.[45]

By the end of the nineteenth century the structure and stereochemistry of the monosaccharides, the basic building blocks, had been deduced through the pioneering work of Emil Fischer.[46] Despite this early work, the understanding of the relationship between carbohydrate structure and biological function has lagged behind that of proteins

and nucleic acids. Before the discovery of the biological importance of glycoconjugates, carbohydrates were only recognized as structural and energy molecules such as cellulose, chitin and starch. The complexity of carbohydrate structures, the difficulty in determining their sequence and their non-template based biosynthesis have contributed to this disparity in knowledge.[47]

In the last twenty years, new technologies developed in the area of Glycobiology,[48] a term first coined by Rademacher, Parekh and Dwek in 1988, have extended our knowledge of carbohydrate structure, biosynthesis and biology. These advances have opened the door for the development of carbohydrate-based therapeutics.[49] Antimicrobial and antifungal drugs are commonly prescribed to fight pathogenic infections; however, due to increased observation of drug-resistant microbes, drug toxicity in patients and drug costs, this treatment method is encountering increasing hurdles. An alternative and promising therapy is the application of carbohydrate-based vaccines for protection against infectious pathogenic agents.

The aim of a vaccine is to elicit a protective, long-lasting immune response against a pathogen in the healthy individual prior to infection. A glycoconjugate vaccine construct consists of a carbohydrate structure, molecular marker, linked to a carrier protein, such as tetanus toxoid. A molecular marker (or hapten) of the pathogen is employed that will prime the host's immune system to target infecting pathogens that display this structure on their outer surface. A vaccine consisting of a small synthetic hapten may be preferable due to the challenges associated with isolating homogenous complex glycans from heterogeneous natural sources. The choice of hapten is important to ensure pathogen-specific, protective and long lasting immunity. Evidence for suitable

structures may be gleaned from studying the specificity of protective antibodies provided by the immune response itself. Understanding the molecular interactions that occur between antibodies and carbohydrates can provide the basic knowledge necessary to tailor carbohydrate-specific therapies.

1.2 Recognition of carbohydrate antigens by antibody binding sites.

1.2.1 Carbohydrate antigens.

Carbohydrate antigens displayed at the surfaces of cells may elicit a powerful immune response. Unfortunate blood transfusion reactions and organ transplant rejections result from responses to carbohydrate antigens with deleterious effects.[50,51] In addition, autoantibodies against host gangliosides generated following infections by *Campylobacter jejuni*, are implicated in the autoimmune disease Guillain-Barré syndrome (GBS), the most frequent cause of acute neuromuscular paralysis.[52] Fortunately in most cases, the immune system has evolved the ability to discriminate foreign molecules (non-self antigens) from the background of self-antigens and is capable of recognizing slight differences in glycan structures or even alterations of glycosylation patterns on host cells.

In some situations, carbohydrate antigens may be deliberately targeted with beneficial effects. For example, during malignant transformation, cells often undergo changes in the quantity and/or type of cell surface carbohydrates that are expressed thus providing a means to recruit the immune system and target cancer cells.[53] Anticancer vaccines based on these tumor-associated carbohydrate antigens (TACAs) or tumor-specific carbohydrate antigens (TSCAs) are currently under development.

Currently, vaccines based on cell-surface capsular polysaccharide structures are being used clinically to provide protective immunity against a number of infectious agents including, for example, *Neisseria meningitidis* (Groups A, C, W135 and Y CPSs, ACWY Vax®), *Salmonella typhi* (Typhim Vi®) and *Streptococcus pneumoniae* (23 CPSs, Pneumovax®).[54] Glycoconjugate vaccines based on the covalent attachment of a cell surface carbohydrate from a micro-organism to a carrier protein are proving to be the most effective means to generate a protective immune response. Three glycoconjugate vaccines, against *Haemophilus influenzae* type b (QuimiHib®), *Neisseria meningitidis* Group C (NeisVac C®) and seven serotypes *Streptococcus pneumoniae* (Prevenar®) are currently licensed. The *H. influenzae* type b represents the first vaccine containing a fully synthetic carbohydrate hapten attached to the carrier protein, tetanus toxoid (Figure 1.2).[55] Due to their great therapeutic potential, cell surface carbohydrate based vaccines against other pathogens are currently under development.[54,56]

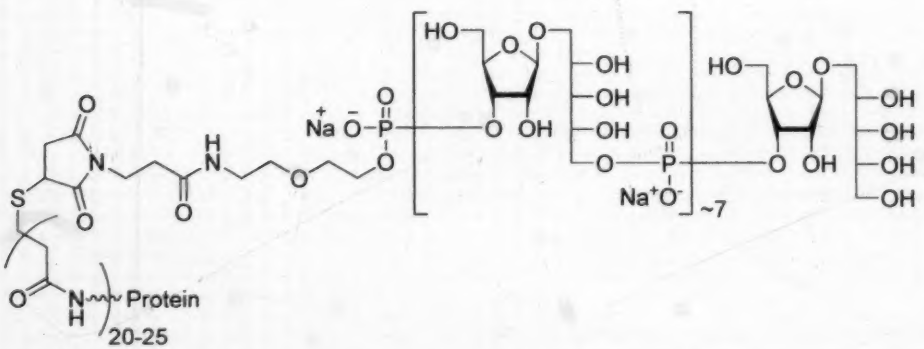


Figure 1.2. *Glycoconjugate vaccine licensed against H. influenzae type b containing the first fully synthetic carbohydrate hapten linked to a carrier protein.*[55]

1.2.2 The immune response to carbohydrate antigens.

The key function of the immune system is to defend the body against a wide variety of pathogenic infectious agents.[57] The immune response is a complex process involving both innate and adaptive mechanisms. Following initial infection, innate immunity provides a formidable front line defence against invading pathogens.[58,59] The elements of innate immunity, including the body's epithelial barrier, macrophages, granulocytes, and dendritic cells, provide a non-specific response to infection. The cells of innate immunity are equipped with sensors called Toll-like receptors, enabling them to recognize certain common structural features of microorganisms termed pathogen-associated molecular patterns; in fact, some of these patterns are carbohydrates such as lipopolysaccharides and β-glucans.[60] When an infection eludes or overwhelms the innate defence mechanisms adaptive immunity is triggered.

The adaptive immune response is an extremely powerful and efficient defence system. It provides the means to generate a rapid, highly specific and often protective response to combat the myriad of potential pathogens. To mount a response against any organism the immune system is equipped with the ability to recognize a wide variety of molecular structures. During a host response to infection the active molecules involved in these immune recognition events against a foreign substance are proteins called antibodies which are produced and secreted by B cells. An antibody is a glycoprotein that binds specifically to a particular substance, its antigen. There are several classes or isotypes of antibodies each with their own properties and effector functions. The production of antibody including isotype is tightly controlled and dependent on the functioning of the two arms of adaptive immunity, the cell-mediated and humoral

responses. Both types of responses involve complex intercellular and intermolecular interactions.

Initiation of either a cell mediated or humoral secondary immune response is critically dependent on the nature of the antigen. Antigens that elicit an immune response are classified as being either T cell dependent or T cell independent. These two classes are distinguished based on the specificity and presentation of certain antigens *via* the major histocompatibility complex (MHC) class II molecule. The MHC class II molecule is found only on a few specialized antigen-presenting cell types, including macrophages, dendritic cells and B cells. The MHC class I molecules are found on almost every cell of the body and present intracellular peptide fragments (e.g. viral proteins) to cytotoxic killer T-cells with recognition of a foreign peptide normally targeting the former cell for lysis. T cell dependent antigens, such as proteins, are processed by antigen presenting cells (i.e. dendritic cells, macrophages and B cells) producing peptide fragments that are bound and presented as a peptide-MHC II complex for recognition by a T cell receptor. Pure carbohydrate antigens typically are not presented by the MHC class II molecule and belong to the class of T cell independent antigens. However, zwitterionic polysaccharides (ZPSs) from the capsules of some bacteria are processed to low molecular weight carbohydrates then presented to T cells through the MHC class II molecule.[61]

Cell-mediated immune response involves activation of a class of thymus derived lymphocytes called T cells. A crucial effect of innate immunity important for an adaptive immune response is the activation and migration of antigen presenting dendritic cells to the lymph nodes.[62] Immature dendritic cells are capable of presenting peptides on their

cell surface *via* the MHC class II molecule for T cell recognition.[63,64] Although recognition of a peptide-MHC II complex on the surface of a dendritic cell by a T cell receptor is a rare event, this activates the naïve T cell and initiates cell-mediated immunity. The stimulated naïve T cell then proliferates and differentiates into one of three armed effector T cells; the cytotoxic killer T cells and either T_H1 or T_H2 cells.[65] Cytotoxic killer T cells are capable of directly attacking invading cells. T_H1 and T_H2 cells function by releasing cytokines to activate macrophages and initiate B cells to differentiate and produce certain antibody isotypes. A successful antibody immune response to T cell dependent antigens, such as proteins, requires close cooperation between stimulated effector T cells and B cells.

Humoral immunity is an antibody-mediated specific response to infection. Bone marrow derived lymphocytes called B cells are the antibody producing cells of the body. Humans possess millions of circulating naïve B cells each of which express immunoglobulin (Ig) membrane bound receptors, termed B cell receptors that have unique antigen specificity for each cell. Each B cell is capable of producing and secreting only one type of antibody with a binding site similar to that of its membrane bound molecule. Protein antigen encountered by a naïve B cell is presented on the outer cell surface in the context of an antigen:MHC class II complex for recognition by an antigen-primed effector T cell. T cell activation of the B cell results in clonal expansion and differentiation into either an antibody-secreting plasma cell or a memory B cell. The plasma cells are involved in the primary immune response which results in maximum antibody levels approximately 10-14 days after infection. The first antibodies detected are predominantly of the low-affinity immunoglobulin M isotype (IgM). Following

subsequent re-exposure to the original antigen, the immune system is able to respond more rapidly because of the existing memory B cells from the primary response; now antibody production is more rapid and peaks approximately 7 days after infection. The memory B cell clones, stimulated by effector T cells, now produce immunoglobulin molecules of the IgG rather than IgM class. Prolonged or repeated exposure to the antigen results in an increase in intrinsic affinity of the IgG antigen binding sites; these higher affinity antibodies evolve from the processes of gene recombination, antigen selection and somatic mutation. Class switch and affinity maturation of Ig are key features of the secondary response mediated by cellular immunity following subsequent infection.

Though pure carbohydrate antigens are not presented by the MHC class II molecule, certain carbohydrate antigens alone can stimulate primary but not secondary B cell responses in the absence of T cell help (Figure 1.3a). T cell independent carbohydrate antigens are typically large polysaccharides with repeating molecular structures capable of cross linking B lymphocyte surface Ig which promotes a primary immune response. The typical responses are characterized by IgM antibody, absence of class switch and affinity maturation and poor immunological memory. T cell independent antigens are divided into two groups. Type 1 antigens, which include cell wall components such as lipopolysaccharides, are capable of activating both immature and mature B cells; whereas, type 2 antigens such as capsular polysaccharides can only stimulate mature B cells.

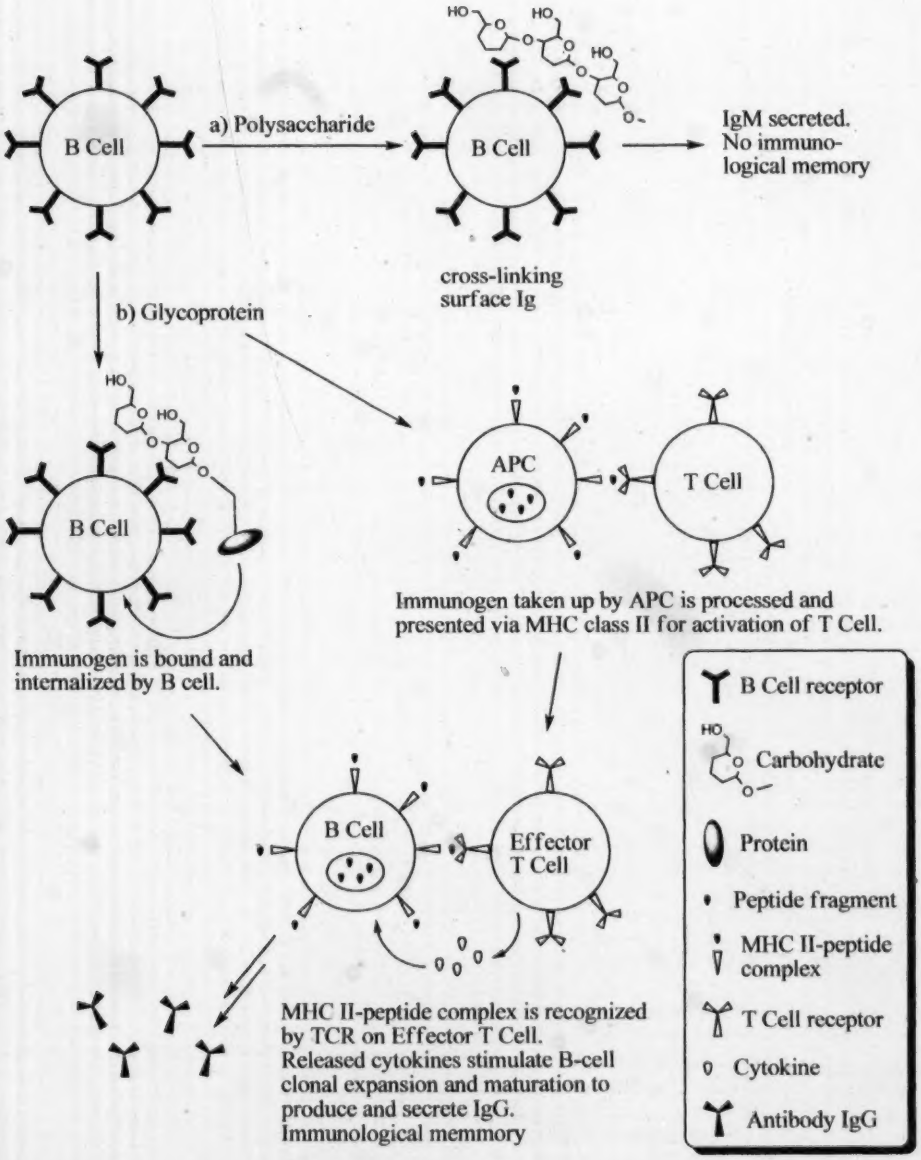


Figure 1.3. *B cell mediated immune response to carbohydrate antigens. a) Response to T-cell independent polysaccharide antigens results in the production of IgM without immunological memory; b) Glycans conjugated to T-cell dependent antigens result in the production of IgG and immunological memory.*

Carbohydrate antigens presented in the context of an intact membrane, such as a liposome, elicit a better primary immune response than do soluble antigens. However, to elicit a secondary immune response against carbohydrate antigens and benefit from Ig class switch, affinity maturation and immunological memory, oligosaccharides must be chemically linked to proteins or peptides, forming a glycoconjugate vaccine (Figure 1.3b). The two portions of the vaccine, the carbohydrate and protein are both necessary for forming a protective, long lasting immune response against a pathogen. The carbohydrate portion of the vaccine is recognized by the B cell receptor, thus secreted antibody will be specific for a carbohydrate epitope. The protein or peptide portion of the vaccine is necessary for T cell activation and elicitation of the secondary immune response. Almost any carbohydrate epitope can induce antibody production using appropriate carriers and adjuvants. The ability to establish a secondary immune response against an oligosaccharide linked to a protein carrier forms the rationale behind the development of synthetic glycoconjugate vaccines.

1.2.3 The antibody molecule.

Antibodies are antigen-reactive glycoproteins present in the immune serum, called anti-serum. They are obtained from a vertebrate host after exposure to a given antigen, called the immunogen. The basic structure of almost all immunoglobulin (Ig) molecules is composed of two identical light (L) polypeptide chains and two identical heavy (H) polypeptide chains, linked together by disulfide bonds.[66] The different polypeptide chains are composed of compact globular domains containing approximately 110 amino acids. The light chains are composed of a variable (V_L) and constant (C_L) domain; whereas, the heavy chains have one variable (V_H) and three or four constant domains

designated, C_{H1}, C_{H2}, C_{H3} (and C_{H4}). To date the *Camelidae*, is the only taxonomic family known to possess functional heavy-chain antibodies, lacking light chains.[67]

The whole Ig molecule has a symmetrical Y-shape with two identical arms that make up the fragments with specific antigen binding (or Fab) which are joined by a flexible hinge to the stem, termed the Fc portion (Figure 1.4). The Fc region contains the paired C_{H2}, C_{H3} (and C_{H4}) domains of both heavy chains. These chains are glycosylated to varying extent by *N*-linked glycans. Antibodies are divided into different classes, called isotypes, based on structural differences in the heavy chain constant regions. In mammals, five classes have been detected: IgA (α), IgD (δ), IgE (ε), IgG (γ) and IgM (μ). There are two types of light chains, kappa (κ) and lambda (λ), which are common to the different Ig classes. The usage of κ and λ varies between species and Ig classes, though no functional differences have been found between antibodies with either light chain. Specific proteases can be used to intentionally partially digest the amino acid sequence of the antibody molecule to produce conveniently sized functioning fragments. The enzyme papain cleaves the antibody molecule into three pieces, two Fab fragments and one Fc fragment. Digestion of the antibody by another enzyme, pepsin, results in one $F(ab')_2$ fragment and many small pieces of the Fc fragment.

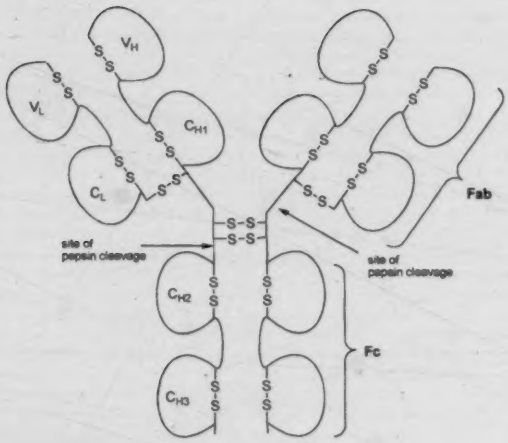


Figure 1.4. *The antibody molecule.*[68]

The major type of immunoglobulin in human or mouse serum is IgG, with lesser amounts of IgM. Some species have subclasses of IgG. In humans, mice and rats there are four subclasses that are numbered based on their decreasing serum concentration (IgG_1-IgG_4); while in rabbits only one has been detected. No subclasses of IgM have been detected. Structurally, IgG antibodies exist as monomers. The IgM antibody possesses a pentameric structure. The antibody Fc region is responsible for mediating the effector functions of the antibody, such as complement activation and interaction with Fc receptors aimed at eliminating antigen-antibody complexes.

Each Fab region contains the V_L and C_L domains of the L-chain and two domains from the H-chain, V_H and C_{H1}. Analysis of the amino acid sequences of the V_L and V_H domains reveal three regions of high variability, termed the hypervariable regions or complementarity determining regions, CDR1-CDR3. These are flanked by four segments with relatively high homology that are termed the framework regions. The framework

regions form β-sheets that provide the structural stability of the entire domain. When the V_L and V_H domains are paired in the antibody, the three CDR's from each chain come together to define the size and shape of a single hypervariable site at the tip of each arm. This site is the antigen-binding site or antibody combining site. There are two identical sites for each IgG molecule and ten for each IgM.

The antibody repertoire, that is, the total number of antibody specificities available to an individual, is estimated to be at least 10^{11}. However, the total number of specificities available to an individual at any one time is limited by the total number of circulating B cells and the antigens encountered. Diversity in antibody specificity is generated from a limited number of inherited V-region gene sequences that code for the binding site domain of an antibody. The limit of antibody diversity, though, is further expanded upon by somatic recombination of the separate gene segments coding for the antibody binding site. These mechanisms for generating diversity take place during the rearrangement of gene segments in the initial development of B cells. An additional source of diversification occurs by somatic hypermutation, which results only when B cells respond to antigen along with signals from activated T cells.

Maturation and clonal expansion of an antigen activated B cell, following infection or immunization, creates multiple cells capable of producing and secreting multiple copies of identical antibody. The antiserum collected from the animal, however, is a mixture of antibody subpopulations, each population with a unique binding site. A collection of polyclonal antibodies is produced in response to the many different epitopes that the host encounters, therefore, polyclonal antibodies are variable and have limited overall specificity. The antiserum collected from separate immunizations will each have

different populations of antibodies with varying overall specificity. Though, this diverse specificity is beneficial to the host mounting an immune response, a source of homogenous antibody is often desired for medical and biochemical purposes. Crystallography and amino acid sequence analysis both require pure antibodies.

1.2.4 Production and purification of monoclonal antibodies.

The development of antibody producing myeloma cells was an important achievement for providing sources of pure antibody. Myeloma is a cancer of antibody-producing cells, and myeloma cell lines are available which multiply rapidly and produce large amounts of antibody. These cell lines arose by spontaneous and random transformation of B cells, thus their antigen specificity is unpredictable and can not be pre-defined. The hybridoma method developed by Köhler and Milstein in 1975 provides a near limitless source of antibody with predefined specificity.[69] This achievement has benefited all of biochemical research, as monoclonal antibodies with almost any specificity can be made available.

Antibodies with a desired specificity can be generated by immunizing a mouse with an antigen of interest. The antibody producing plasma cells isolated from the spleen can be fused with selected myeloma cells incapable of producing their own antibody. The resulting hybridomas are grown in HAT culture medium containing hypoxanthine, aminopterin and thymidine.[70] Un-fused myeloma cells lacking hypoxanthine-guanine phosphoribosyltransferase, an important enzyme of the nucleotide salvage pathway catalyzing the synthesis of inosine monophosphate (IMP) from hypoxanthine, can not survive in the culture medium. Thus, the fused hybridomas, having inherited longevity from the myeloma cells, are each capable of producing monoclonal antibodies with

unique antigenic specificity. Antibody produced by the hybridoma colonies is then screened against the antigen of interest. Antigen-positive cultures can then be further cloned and grown in culture to provide a reliable system for the production of antibody with desired specificity.

1.3 The thermodynamics of carbohydrate-protein interactions

1.3.1 Intermolecular forces and the association constant.

Antigen-antibody complex formation involves reversible formation of multiple non-covalent bonds. The portion of the antigen to which the antibody combining site is complementary is referred to as the epitope or antigenic determinant.[71] Thus, the epitope is a three dimensional area on the surface of the antigen and is functionally described as the area that cannot be modified without affecting binding. A caveat being, that although an analogue of an antigen may contain the required epitope, unfavourable steric interactions between the analogue and antibody remote from the binding site may lead to decreased affinity and loss of binding. Due to the amphiphilic nature of carbohydrates a variety of forces are involved in carbohydrate-protein interactions.

A quantitative treatment of antigen-antibody association can reveal important attractive and repulsive forces that govern the strength and specificity of the binding event. The association of a single antigen (Ag) and antibody combining site (Ab) at equilibrium is described by equation (1):

$$Ag + Ab \rightleftarrows Ag{:}Ab \qquad (1)$$

The association constant K_A describes the position of the equilibrium (eq. 2), where [Ag] and [Ab] are the equilibrium concentrations of unbound antigen and antibody and [Ag:Ab] is that of the bound complex:

$$K_A = 1 / K_D = [Ag:Ab] / [Ag]\,[Ab] \qquad (2)$$

The association constant can be related to the Gibbs free energy (eq. 3) to provide a quantitative measure of the binding strength, where R is the universal gas constant (1.987 $cal \cdot K^{-1} \cdot mol^{-1}$) and T is the absolute temperature. For a given antigen-antibody complex, the binding strength is termed the affinity. Partitioning of the free energy (ΔG) into enthalpic (ΔH) and entropic (ΔS) terms reveals the relative contributions attractive forces and changes in molecular order, particularly solvent reorganization, have on complex formation:

$$\Delta G = \Delta H - T\Delta S = -RT ln K_A \qquad (3)$$

1.3.2 Thermodynamic parameters of binding.

The enthalpic contribution accounts for changes in the attractive and repulsive forces between antigen, antibody and solvent during complex formation in solution (eq. 4).

$$\Delta H = \Delta H_{bind} + \Delta H_{solv} \qquad (4)$$

The favourable attractive forces (ΔH_{bind}) between non-bonded species are referred to as cohesive or non-covalent forces which individually are typically weak in comparison with covalent bonds. Cumulatively, a large number of these weak attractive

interactions can result in a larger total binding energy. The non-covalent forces between carbohydrate antigens and antibodies can be non-specific (i.e. van der Waals or dispersive forces) or specific (i.e. hydrogen bonds).

An intra- or intermolecular hydrogen bond can form between the lone electron pair of an acceptor **B** (typically oxygen, nitrogen or sulphur) and a hydrogen of a donor (**H-A**), where **A** is an electronegative atom such as oxygen or nitrogen. Formation of hydrogen bonds is dependent on the orientation of the interacting partners. The strongest interaction exists if the hydrogen lies along or near the straight line between **A** and lone pair electrons of **B**. The energy of a hydrogen bond has been estimated to be within 1.0-4.0 kcal·mol^{-1}. Hydrogen bonds between uncharged groups have been shown to range from 1.0-1.4 kcal·mol^{-1}, while the energy of a hydrogen bond between a charged donor and acceptor is ~4 kcal·mol^{-1}. Hydrogen bonds are important in conferring the specificity and affinity of carbohydrate-protein interactions. The many polar hydroxyl groups of carbohydrates are involved in hydrogen bonding interactions with polar groups on the amino acid side chains of the antibody and water molecules embedded within the binding site.

Dispersive forces resulting from interactions between temporary dipoles are important contributors to overall interaction energies. These forces exist between all complexed molecules but are emphasized for solutes (or areas) with increased polarizability, such as lipophilic surfaces. The strength of the interaction is generally proportional to the surface area and the dependence on distance helps orient the ligand during binding.

In aqueous solution, antigen and antibody are both solvated forming hydrogen bonds, and other polar interactions with water. During complex formation some of these bonds with water are lost and new ones are formed. The enthalpy of solvation (ΔH_{solv}) is the change in energy that results when two species are separated forming bonds with the solvent.

In general, for complex formation antigens and antibodies in solution have to overcome large entropic barriers. During the binding event each component experiences changes in free rotation (ΔS_{rot}) and translation (ΔS_{trans}). As well portions of the molecules will each lose conformational freedom (ΔS_{conf}). However, a significant amount of entropy is gained when the solvated molecules combine as organized water molecules are displaced from their surfaces (ΔS_{solv}).

$$\Delta S = \Delta S_{rot} + \Delta S_{trans} + \Delta S_{conf} + \Delta S_{solv} \qquad (5)$$

1.3.3 Quantification of carbohydrate-protein interactions.

Historically, the precipitin assay developed by Michael Heidelberger was the first method used to quantify antigen-antibody interactions. This method relied upon the visible formation of a precipitate consisting of large aggregates of antigen cross-linked by antibody molecules. Data obtained from the application of this method to systems of polysaccharide antigens and antibodies showed for the first time that the reactions obeyed the laws of mass action and that the reactants were multivalent.[72]

Today the most frequently used method for studying carbohydrate-antibody interactions is the enzyme-linked immunosorbent assay (ELISA). ELISA is a precise solid-phase assay which measures antigen-antibody interaction by the detection of an

enzyme catalyzed coloured response. Ligand or receptors coated onto a solid support, such as a microtitre plate, are treated with solutions containing their complimentary binding partner. The soluble reactant can be conjugated to a reporter enzyme enabling direct detection of the immune reaction or alternatively, indirect detection can be used employing a third reactant, commonly an enzyme-linked antibody that binds to the soluble antibody recognizing the solid supported antigen. Competitive ELISA assays can be introduced by addition of increasing concentrations of a soluble antigen that will compete and reduce the amount of labelled reactant binding to the solid support. This provides a convenient means of comparing the binding of analogues with native antigens.

Other methods that are commonly used to measure protein-carbohydrate interactions include surface plasmon resonance (SPR), isothermal titration calorimetry (ITC) and frontal affinity chromatography with mass spectrometry detection (FAC-MS). Functional group replacement can also be coupled with nano-ES-FT-ICR MS or an additional technique termed blackbody infrared radiative dissociation (BIRD) using Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry to map the location and importantly strength of hydrogen bonds.[73,74]

1.3.4 Chemical mapping of carbohydrate epitopes.

The method of choice for obtaining detailed three-dimensional structural information about the antibody oligosaccharide complex is protein crystallography; however, this relies heavily on antigen-antibody Fab co-crystals. To date only a few structures of oligosaccharide-antibody Fab complexes have been solved.[75-83] A large number of Fab fragments without antigen have been solved to high resolution and although these, unfortunately, lack the desired information on antigen-antibody

interactions, computational methods can provide some structural insight allowing ligands to be docked into the binding site.[84]

In the absence of protein structural data, information about the antibody binding site can be obtained indirectly by establishing the structural features of the carbohydrate antigen which permit complex formation. In the early development of immunochemistry, Karl Landsteiner discovered the important concept that a low molecular weight hapten, possessing an identical or related specific determinant to that on an antigen, would competitively inhibit antibody from reacting with antigen.[85] This important observation established the relationship between chemical structure and immunological specificity eventually leading others to speculate about the size and topology of the antibody binding site.[71] The antigenic polysaccharide dextran and the availability of isomaltose oligosaccharides of varying length provided Kabat with an ideal system for estimating the size of an antigenic determinant.[86,87] Studies found that, although a heterogeneous population of antibodies was obtained from immunization with dextran, an upper limit was reached with isomaltose oligosaccharides of increasing chain length in inhibiting dextran-antidextran precipitation, up to a hexa- or heptasaccharide. Though this revealed for the first time the size of an antibody combining site, Kabat admittedly acknowledged that because the conformation of the flexible oligosaccharides was unknown these studies did not provide knowledge of the shape or topology of the antigenic determinant or antibody binding site.

Systematic variation of each position of the carbohydrate hapten structure and a knowledge of the relative inhibiting power of these analogues provides a valuable tool to discern the area within the antigenic determinant and the forces contributing to

association.[88-90] Early work in this area was limited to the use of naturally occurring mono- and oligosaccharides or chemically modified simple sugars.[91,92] More complex structures became available with advances in carbohydrate synthesis. Along these lines, several strategies have been devised to identify hydroxyl groups of a carbohydrate ligand forming hydrogen bonds essential for antigen-antibody binding.

Lemieux developed the use of mono-deoxy analogues in combination with conformational analysis to provide a three dimensional model of the topography of the oligosaccharide epitope.[93,94] The key hydroxyl groups forming hydrogen bonds within the protein binding site could be determined from the relative inhibitory power of the deoxy analogues (Figure 1.5a-e). Removal of an essential hydroxyl group would result in a marked decrease in inhibitory power compared to the natural hapten. Deoxygenation of a hydroxyl group located at the periphery of the binding site or exposed to bulk solvent would not affect the binding of the mono-deoxy analogue appreciably. To further distinguish these latter hydroxyl groups Lemieux employed mono-*O*-methyl analogues.[89] Methylation could discriminate hydroxyl groups located at the periphery of the oligosaccharide-protein complex, since these show substantially reduced binding, due to the steric bulk of the methyl group. The binding activity of methylated hydroxyl groups exposed to bulk solvent remained unchanged.

Hydroxyl groups within the antibody binding site can conceivably serve a hydrogen bond acceptor and/or donor function. Deoxygenation of a hydroxyl group located within the binding site eliminates both acceptor and donor capabilities. To evaluate the hydrogen bond acceptor functions in an oligosaccharide, Glaudemans has substituted hydroxyl groups with fluorine (Figure 1.5f).[88] Evidence from crystal data of

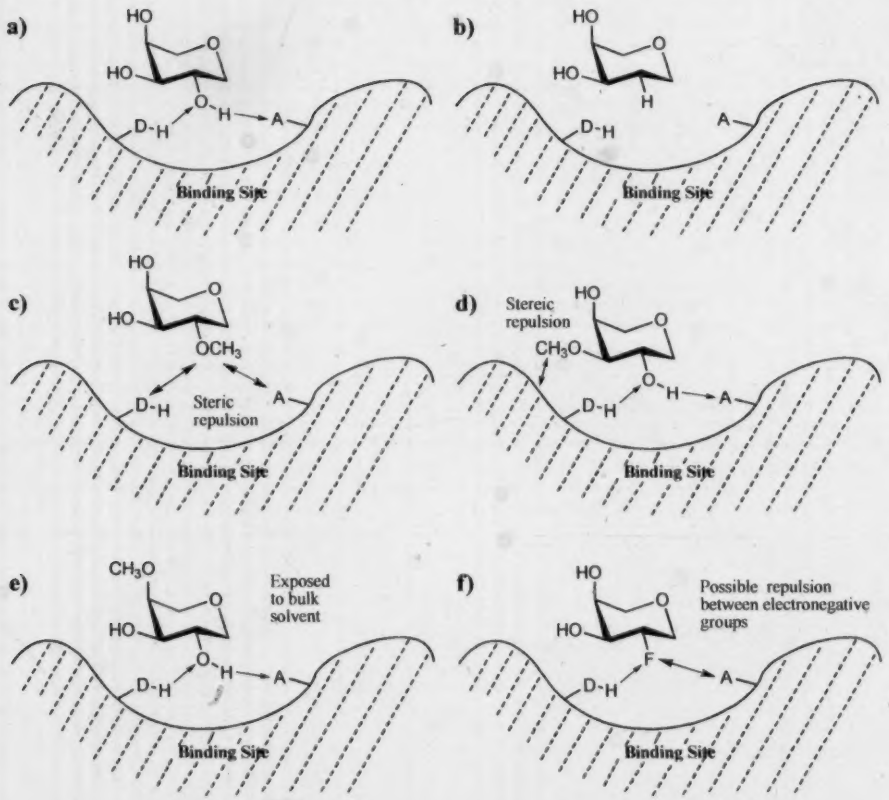


Figure 1.5. *Strategies to chemically map the role of oligosaccharide hydroxyl groups in protein complexes. (a) Saccharide hydroxyl groups can serve as both hydrogen bond acceptors and/or donors with the protein. (b) Replacement of the hydroxyl group with a deoxy function abolishes hydrogen bond acceptor and donor functions. (c) Methyl ethers can distinguish between hydroxyl groups that are located at the periphery of the binding site or solvent exposed. Hydroxyl groups that are located within the binding site become inactive when methylated. (d) Hydroxyl groups at the periphery of the binding site, involved in interactions with the protein yet are partially solvent exposed, experience a large decrease in activity when methylated. (e) Methylation of hydroxyl groups that are exposed to the solvent show little or no change in binding strength. (f) Replacement of a hydroxyl group with a fluorine atom may preserve a hydrogen bond acceptor function.*

small molecules indicated that though fluorine forms weak hydrogen bonds it could serve as a hydrogen bond acceptor and in principle preserve a hydrogen bond acceptor role in an oligosaccharide-protein complex.[95] To confirm the hydrogen bond acceptor role of a

hydroxyl, inhibition data from the mono-deoxy fluoro analogues is compared with the activity of the corresponding mono-deoxy analogue.

Differences between the amounts of modified analogue and a reference oligosaccharide required for 50% inhibition (IC_{50}) at a given temperature relate differences in the free energies of binding:

$$\Delta(\Delta G) = \Delta G_{inh} - \Delta G_{ref} = -RT(\ln K_{inh}/K_{ref}) \qquad (6)$$

The relative association constants K_{inh} and K_{ref} are determined from the IC_{50} values for the analogue and reference oligosaccharide, respectively. In terms of the effect of a modification on binding, Lemieux interpreted influences on $\Delta(\Delta G)$ values of 0-0.8 kcal/mol as having a marginal effect on complex stability.[94] A modification which resulted in a $\Delta(\Delta G)$ value >2.3 kcal/mol was considered an essentially inactive compound with an IC_{50} value at least 50 times worse than the reference oligosaccharide. The inactivity of the analogue was indication that the modified hydroxyl group was directly involved in a polar interaction essential for the binding of the reference oligosaccharide to the protein. Modifications resulting in intermediate values of $\Delta(\Delta G)$ (0.8-2.3 kcal/mol) were taken as indicating the hydroxyl group was involved in complex formation, although, the polar interaction was not deemed crucial to stability. This strategy was successfully applied to map the epitope contained in the tetrasaccharide of the Lewis b (Le^b) blood group antigen which is bound by lectin IV from *Griffonia simplicifloria*.[93,94,96,97] The predicted three-dimensional model, based on inhibition data using a series of mono-deoxy and mono-*O*-methyl Le^b analogues to inhibit the binding of native Le^b to lectin IV in a competitive solid-phase radioimmunoassay, was in good

agreement with that subsequently identified for the bound form present in a crystal structure of the lectin-tetrasaccharide complex.[98,99]

In addition to the potentially unfavourable enthalpic cost to binding incurred with removal of a hydroxyl group making a key polar contact, it has been suggested that certain modifications may also alter the solution conformation of an oligosaccharide and subsequently decrease binding by incurring an entropic penalty.[100] Kishi found that decreases in binding of lectin I (*Ulex europaeus*) to structurally modified *C*-glycoside trisaccharide analogues of the H type II blood group determinant largely correlated with changes in analogue solution conformation resulting from modification and suggested that loss of inhibitor activity was an entropic effect. The inhibition data from modified *C*-glycoside analogues was comparable to the complementary *O*-glycosides and it was inferred that alterations of binding affinity in the latter were also entropic; however, conformational analysis was solely based on the structures of the *C*-glycosides and not the *O*-glycosides. It has been shown that there are clear differences in the conformational behaviour of *C*- and *O*-glycosides including increased flexibility in the former;[101] therefore, inferring changes in solution conformation of modified *O*-glycosides from *C*-glycosides may not be valid. However, deductions of the binding-site specificity and details of the binding process from only the relative inhibitory power or ratio of binding constants of related ligands should be made cautiously as the separate contributions of enthalpy and entropy to binding are not considered.

Chemical mapping of carbohydrate epitopes using systematic evaluation of mono-deoxy and mono-*O*-methyl analogues has been employed for systems involving binding to monoclonal antibodies,[102-105] lectins,[96,106,107] and enzymes.[108-111] In general only a few

hydroxyl groups of an oligosaccharide serve critical roles in determining the binding affinity and specificity.

1.4 *Candida albicans.*

1.4.1 Background.

Candida albicans is a dimorphic commensal fungus of humans that commonly inhabits the gastrointestinal tract, oropharyngeal cavity and vulvovaginal tract.[112,113] Two serotypes of this species have been defined, designated as A and B.[114,115] Under appropriate conditions the normally commensal host-fungal interaction can become pathogenic and result in the development of an opportunistic candidal infection, or candidiasis. Infection may manifest as either cutaneous/mucocutaneous candidiasis or hematogenously disseminated disease. The former, referred clinically and historically as thrush involves the mucosa of the oral cavity or vaginal tract and generally is not considered directly life-threatening. Bloodstream disseminated candidiasis, is often life-threatening and involves deep organ systems, including the kidney, liver, spleen, heart, and lung and may extend to the central nervous system. Individuals most at risk for serious infection are immunocompromised patients and those undergoing long-term antibiotic treatment.[116] Studies monitoring surgical and neonatal intensive care units found that *Candida* species were the fourth leading cause of nosocomial bloodstream infections; over half of the candidiasis infections were attributed to *C. albicans.*[117] Therapeutics to combat these infections is limited to azole derivatives and amphotericin B. However, the toxicity, emergence of resistance and cost of these antifungal agents are potential problems and highlight the need for alternative treatment strategies. A vaccine

against *Candida* appears to be a promising alternative and its development is attracting much attention.[112,118]

1.4.2 The phosphomannan complex of *Candida albicans*.

The cell wall of *C. albicans* is composed largely of carbohydrates (80-90%) with the main constituent being a highly branched glucan polymer.[119,120] Chitin, a minor cell wall component, cross-links and provides support to the glucan framework. Together the glucan and chitin form a rigid wall that provides integrity, shape and importantly shields the fungus against osmotic, chemical and biological harm. Mannan glycoconjugates, glycoproteins and glycolipids, embedded in the outer surface are a major component of the cell wall.[121] There has been considerable interest in the mannan portions of the cell wall as a component involved in pathogenesis of candidiasis and as a potential target for the development of therapeutics. Studies have revealed that the surface mannan are highly immunogenic[122] and function as adhesins responsible for attachment of *C. albicans* to splenic and lymph node macrophages.[123-126]

The outer cell wall is composed of a complex mixture of mannoproteins composed of both *O*-linked and complex *N*-linked structures.[121] The *O*-linked mannan consists mostly of short (1→2)-α-D-mannopyranan chains, though, it has been suggested that *O*-linked chains with 4 or 5 mannose units may have β-linked mannose at the reducing end.[127] The structure of an *N*-linked phosphomannan protein associated with the adhesin and immunogenic properties has been elucidated using NMR and mass spectrometry.[128-134] The phosphomannan portion is heterogeneous and differences in glycoforms have been shown to depend on serotype, morphology and growing conditions (Figure 1.6). Extraction of yeast cells with β-mercaptoethanol gives a phosphomannan

complex composed primarily of mannan with about 3.5% protein.[135] The mannan structure consists of an (1→6)-α-linked backbone with oligomannose side chains containing (1→2)-α- and (1→3)-α-linked residues with some branching of these chains with (1→6)-α-linked mannose residues. These mannan chains are further extended with three types of (1→2)-β-linkage-containing manno-oligosaccharides. Two forms of the (1→2)-β-linked oligosaccharides exist as extensions of the (1→2)-α- and (1→3)-α-linked chains and corresponds to the serotype-A specific epitope of *C. albicans*. A third structure, which can be released by treatment of the mannan with weak acid, is located in a phosphodiesterified oligosaccharide moiety found as a common epitope of *C. albicans* serotypes A and B. The (1→2)-β-mannans released by acid range in length from a single residue up to a heptamer. A phospholipomannan containing β-mannans has also been identified in both serotypes of *C. albicans*.[136]

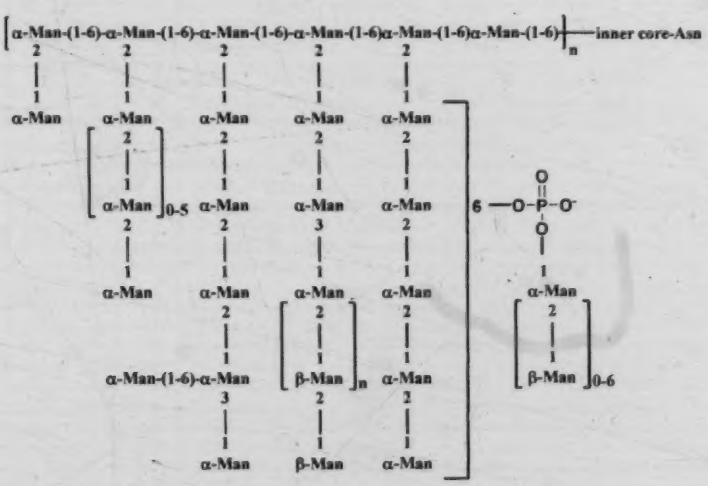


Figure 1.6. *The phosphomannan complex.*

1.4.3 Monoclonal antibodies specific for the PMC.

Mice immunized with whole yeast cells yielded a non-protective monoclonal IgM antibody, labelled B6.[135] MAb B6 was found to be specific for an epitope contained in the acid stable portion of the PMC. Vaccination of mice with liposome encapsulated PMC adhesin extract was also found to elicit an antibody response.[135,137,138] However, the PMC alone was poorly immunogenic.[112] Antiserum from animals vaccinated with the liposome-PMC formulation increased resistance to disseminated candidiasis in passively immunized mice. Two monoclonal antibodies, an IgM and IgG3, labelled B6.1 and C3.1 respectively, were isolated from mouse antiserum.[137,138] Both antibodies were found to agglutinate *C. albicans* cells and provide protection in mice from disseminated candidiasis. Immunofluoresence confocal microscopy indicated that the epitope recognized by the protective antibodies is present over the entire surface of the yeast. Agglutination studies suggested that both antibodies were specific for an epitope associated with the acid-labile portion of the PMC, namely a 1,2-β-mannan trisaccharide. Bundle and Nitz employed synthetic 1,2-β-mannan oligomers from di- to hexasaccharides as inhibitors of mAb C3.1 and B6.1 in ELISA inhibition studies and found that maximum activity was reached with di- and trisaccharides and diminished significantly for tetra-, penta-, and hexasaccharides (Table 1.1.).[139] This is in marked contrast to the paradigm reported by Kabat wherein homo-oligomers of dextran exhibit increasing activity with increasing size up to a hexa- or heptasaccharide.

Table 1.1. *Inhibition by synthetic (1→2)-β-D-mannopyranoside oligosaccharides of the binding of mAb C3.1 (IgG) to C. albicans PMC.*[139]

Inhibitor†	IC_{50} μmol/L	Relative Potency %
Disaccharide	8	100
Trisaccharide	16	50
Tetrasaccharide	84	10
Pentasaccharide	421	2
Hexasaccharide	844	1

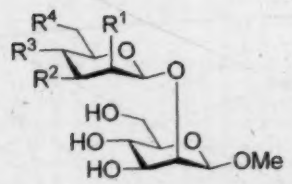
† (1→2)-β-D-mannopyranoside oligosaccharide.

1.5 Scope of project

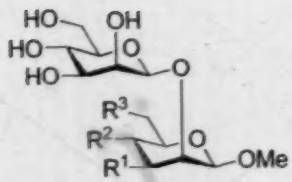
The (1→2)-β-D-mannopyranoside of the phosphomannan complex (PMC) of *C. albicans* represents an intriguing candidate as a hapten for a synthetic conjugate vaccine. Protective monoclonal antibodies raised against the PMC were found to recognize the (1→2)-β-D-mannopyranoside portion. The objective of this project is to provide a detailed description of the size and topology of the protective epitope contained in the (1→2)-β-D-mannopyranoside of *C. albicans* that is recognized by mAb C3.1. Knowledge of the recognition elements of the protective epitope will provide important insights into the minimum sized hapten that can be employed in a synthetic conjugate vaccine. The strategy of chemical mapping, devised by Lemieux, is used to define the key polar contacts, intermolecular hydrogen bonds, required for binding of the sugar to the antibody. Previous, studies found that mAb C3.1 recognized (1→2)-β-D-manno disaccharide and trisaccharide with higher affinity than the higher molecular weight oligomers, thus, in the present study synthetic analogues were limited to a disaccharide or

trisaccharide. Chapter 2 describes the synthesis of a series of mono-deoxy and mono-*O*-methyl disaccharide and trisaccharide analogues of (1→2)-β-D-mannopyranoside (Figure 1.7). Evaluation of the analogues as inhibitors in ELISA inhibition studies is described in chapter 3. The extent each hydroxyl group is involved in the binding of **1** to mAb C3.1 was assessed by comparison of the inhibition data obtained for the modified analogues.

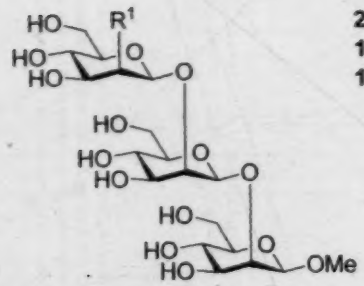
Measurements of saturation transfer difference nuclear magnetic resonance (STD-NMR) spectroscopy for trisaccharide **2** and mAb C3.1 is described in chapter 4.



1 R^1 = OH, R^2 = OH, R^3 = OH, R^4 = OH;
3 R^1 = H, R^2 = OH, R^3 = OH, R^4 = OH;
4 R^1 = OCH_3, R^2 = OH, R^3 = OH, R^4 = OH;
5 R^1 = OH (*gluco*), R^2 = OH, R^3 = OH, R^4 = OH;
6 R^1 = OH, R^2 = H, R^3 = OH, R^4 = OH;
7 R^1 = OH, R^2 = OCH_3, R^3 = OH, R^4 = OH;
8 R^1 = OH, R^2 = OH, R^3 = H, R^4 = OH;
9 R^1 = OH, R^2 = OH, R^3 = OCH_3, R^4 = OH;
10 R^1 = OH, R^2 = OH, R^3 = OH, R^4 = H;
11 R^1 = OH, R^2 = OH, R^3 = OH, R^4 = OCH_3;



12 R^1 = H, R^2 = OH, R^3 = OH;
13 R^1 = OCH_3, R^2 = OH, R^3 = OH;
14 R^1 = OH, R^2 = H, R^3 = OH;
15 R^1 = OH, R^2 = OCH_3, R^3 = OH;
16 R^1 = OH, R^2 = H, R^3 = H;
17 R^1 = OH, R^2 = OCH_3, R^3 = OCH_3;



2 R^1 = OH;
18 R^1 = OCH_3;
19 R^1 = OH (*gluco*);

Figure 1.7. *Synthetic deoxy and O-methyl analogues of disaccharide* **1** *and trisaccharide* **2**.

Chapter Two

Synthesis of methyl (1→2)-β-D-mannopyranoside analogues.

2.1 Introduction: Synthetic aspects of carbohydrate chemistry.

The chemical synthesis of oligosaccharides is an important means for providing essential tools for glycobiology. However, compared to other biopolymers (i.e. proteins and DNA) carbohydrates present a more challenging synthetic problem. In terms of potential structural complexity, the combination of any three amino acids (or nucleotides) results in 6 potential linear products and by comparison, considering regiochemistry and anomeric stereochemistry alone, combination of three D-glucose residues gives 32 unique linear trisaccharides. The number of potential carbohydrate products rapidly increases if branching and stereochemistry of non-anomeric centres are considered. An efficient synthesis of a particular carbohydrate target requires consideration of the important factors controlling both stereoselectivity and regioselectivity:

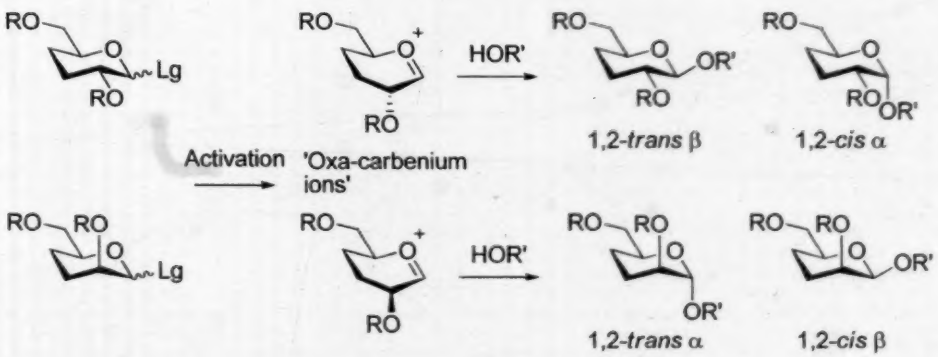
1.) Stereoselectivity: The anomeric effect and neighbouring group participation both have important control over anomeric stereochemistry during glycoside formation where, both α- and β-isomers can form generating diastereomers.

2.) Regioselectivity: A simple monosaccharide has multiple hydroxyl groups that are potential sites for glycosylation. Synthesis of a disaccharide requires multiple chemical steps to protect hydroxyl groups, leaving one hydroxyl available for reaction with a glycosyl donor. Thus, protecting groups play an essential role in synthetic carbohydrate chemistry and an efficient synthetic strategy relies on a minimal number of high yielding, regioselective protecting group manipulations.

2.1.1 Glycoside formation.

A logical retro-synthesis of glycosides involves disconnection of the bond between the anomeric acetal carbon and exocyclic oxygen of the aglycone. Indeed, the most common methods for glycosidic bond formation involve activation then loss of an anomeric leaving group followed by nucleophilic attack at the anomeric centre by oxygen of an acceptor substrate.

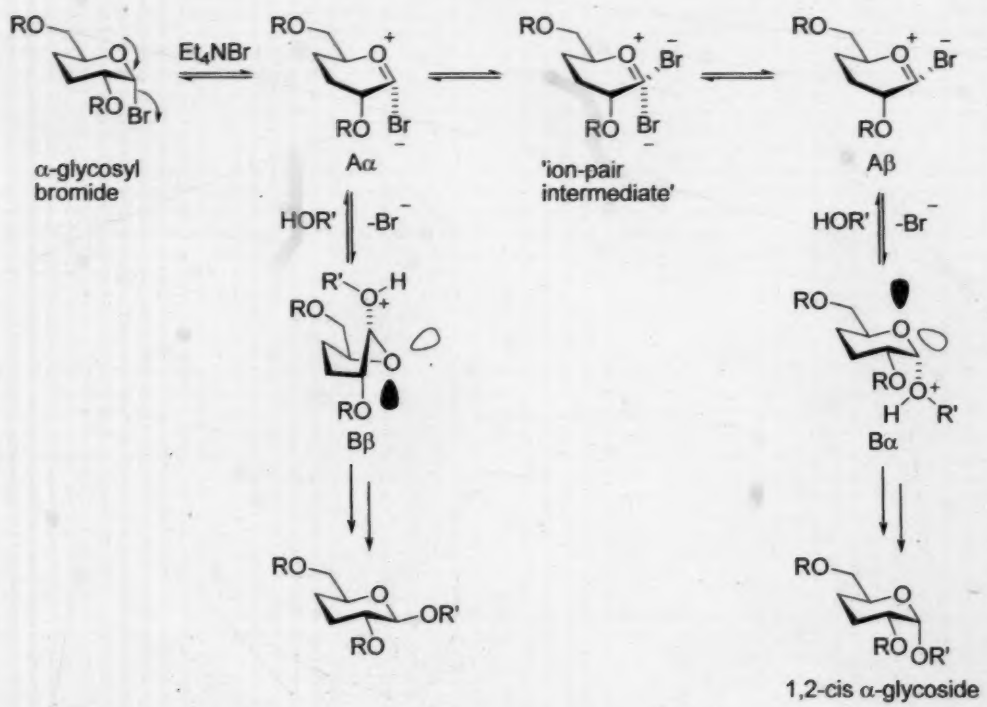
Activation then loss of the leaving group from a glycosyl donor produces a high-energy intermediate termed the oxo-carbenium ion (Scheme 2.1). In a general glycosylation, nucleophilic reaction of the incoming (glycosyl) acceptor (HOR') at the anomeric carbon can occur on either the top or bottom face. Considering the stereochemistry at C-2 of the donor there are four theoretical outcomes for formation of the glycosidic linkage. For discussion purposes, it is common to divide these glycosidic linkages in to three classes based on the configuration of hydroxyl groups at C-1 and C-2 (i.e. the 1,2-configuration): 1,2-*trans* α and β; 1,2-*cis* α; and 1,2-*cis* β. The designation of anomers as α or β is based on the configurational relationship between the anomeric centre and the highest numbered carbon atom of the group of stereocentres. In the Fisher projection of the α-anomer, the anomeric exocyclic oxygen is formally *cis* to the oxygen attached to the reference atom. In the β-anomer the oxygens are *trans*.



Scheme 2.1. *Synthesis of the different glycosidic linkages.*

Historically, halides have played an important role as glycosyl donors in the development of synthetic carbohydrate chemistry.[140] The halide-ion catalyzed glycosidation reaction developed by Lemieux,[141] was a major achievement (Scheme 2.2). For the first time a reliable method was available for the controlled preparation of 1,2-*cis* α-glycosides. Glycosidation involves initial "*in situ* anomerization" of an α-glycosyl bromide to the β-anomer through an ion-pair intermediate. The highly reactive β-bromide then rapidly reacts with the incoming nucleophile to yield the α-glycoside product.

Today, there is an increasing number of leaving groups and activation methods available for glycosylation.[142] However, in the majority of oligosaccharide syntheses three leaving groups are most commonly relied upon: trichloroacetimidates, thioglycosides and glycosyl sulfoxides (Scheme 2.3).

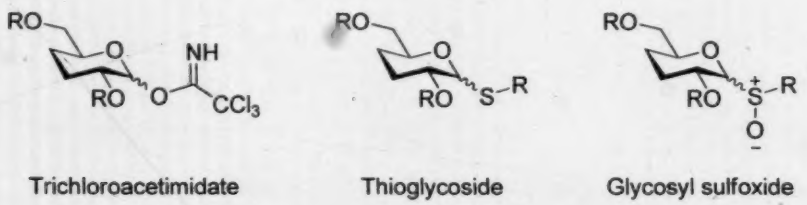


Scheme 2.2. *Halide-ion catalyzed glycosidation.*

Trichloroacetimidate donors, first introduced by Schmidt,[143,144] are prepared from the free-sugar under mild conditions (DBU/CH_2Cl_2) which are compatible with most protecting groups. Typically, glycosylations with trichloroacetimidate donors involve activation using catalytic trimethylsilyl triflate (TMSOTf).[145,146]

Thioglycosides[147,148] are extremely versatile donors, since they are stable to most protecting group manipulations, they can also act as anomeric blocking groups until activated to a leaving group. Several thiophilic reagents are routinely used for activation of thioglycosides, including NIS/TfOH,[149] DMTST (dimethyl(methylthio)sulfonium triflate),[150] IDCP (iodonium dicollidine perchlorate),[151] Ph_2SO/Tf_2O[152] and dimethyl

disulfide/Tf_2O.[153] Kahne introduced glycosyl sulfoxides,[154] which proved valuable for glycosylations of some very unreactive acceptors under mild conditions (Scheme 2.3). Mild oxidation of thioglycosides with *m*CPBA gives glycosyl sulfoxides.



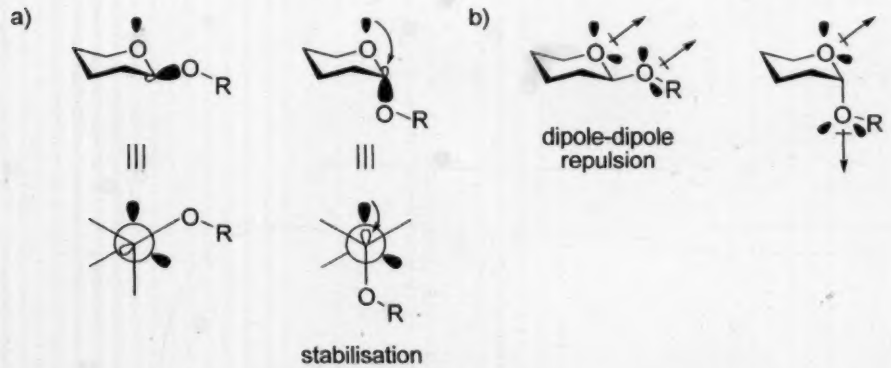
Scheme 2.3. *Common glycosyl donor leaving groups.*

2.1.2 Factors controlling anomeric stereochemistry during glycoside formation.

2.1.2.1 Anomeric effect.

The anomeric effect, first observed by Edward[155] and named by Lemieux,[156] is one of the most important effects controlling stereochemistry of reactions occurring at the anomeric center of sugars. Typically, a substituted cyclohexyl ring adopts one of two possible chair conformations having the greater number of substituents oriented in equatorial positions to minimize steric interactions resulting from 1,3-diaxial interactions. One might expect then that in the pyranose ring the aglycone would also prefer to occupy the equatorial position. However, in general compounds with axial oriented polar substituents such as alkoxy, acyloxy and halide groups are more stable than equatorial ones. The preference for the formation of the axial anomer was termed the anomeric effect and the two most accepted rationalizations are (Scheme 2.4): (1) delocalization of the "axially" located lone-pair electrons of the ring oxygen interacting with the periplanar

C-X bond antibonding σ* orbital in an axial positioned glycoside, and (2) unfavourable dipole-dipole interactions between the carbon-oxygen bond on the ring and the bond from the anomeric carbon to the equatorial heteroatom.

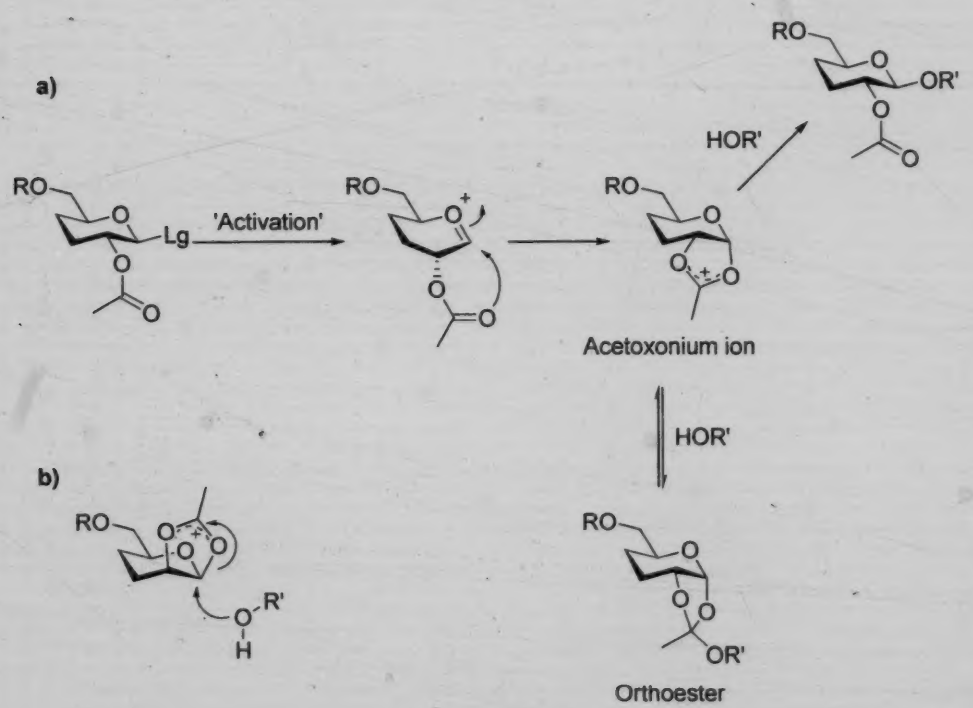


Scheme 2.4. *Rationalizing the anomeric effect. a) Stabilization of the axial anomer by delocalization of lone-pair electrons interacting with anti-bonding molecular orbital (σ*); b) Unfavorable dipole-dipole interactions in the equatorial anomer.*

2.1.2.2 Neighbouring group participation.

An interesting and important effect on stereochemistry in a substitution reaction is the possible participation of a functional group from a neighbouring or more remote centre. In 1942, while studying the substitution of bromide with acetate in several acetoxybromo substrates, Winstein and Buckles, observed a high retention of configuration.[157] To account for the conserved retention they were the first to propose the participation of a neighbouring acetoxy group at the substitution centre during reaction. The most common methods for the synthesis of 1,2-*trans* α- and β-glycosides take advantage of neighbouring group participation to control anomeric stereoselectivity by employing donors with 2-*O*-acyl groups (Scheme 2.5). Activation of the glycosyl donor, usually under acidic conditions, results in loss of the anomeric leaving group to form the

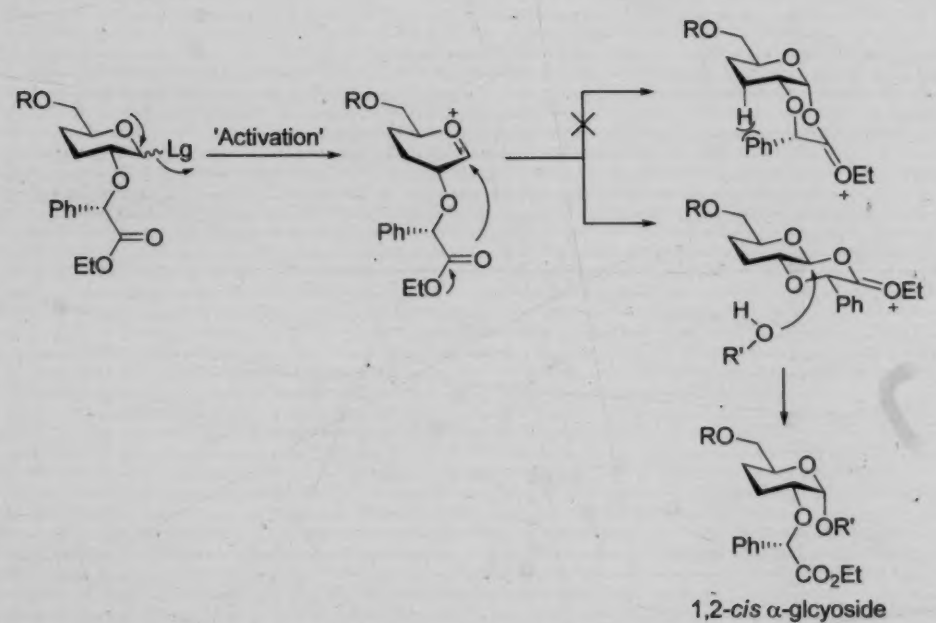
oxo-carbenium intermediate. Subsequent attack by the carbonyl oxygen of the 2-*O*-acyl group on the α-face (as shown in Scheme 2.5a) forms the acetoxonium ion intermediate. Reaction of the incoming nucleophile with the resonance stabilized carbon centre of the acetoxonium forms the corresponding orthoester. Under the acidic glycosylation conditions, the orthoester, in equilibrium with the acetoxonium intermediate, can rearrange to form the glycoside. Overall, the incoming nucleophile is required to react at the anomeric position *trans* to the C-2 substituent resulting in stereospecific formation of the *trans* glycoside.



Scheme 2.5. *Neighbouring group participation. a) Formation of the 1,2-trans β-glucoside. b) Formation of the 1,2-trans α-mannoside.*

The stereoselective synthesis of 1,2-*cis* α-glycosides is a greater challenge than the *trans* linkages. Achievements in this area have been the subject of a review.[142] Recently, Boons,[158-160] has developed a 1,2-*cis* α-glycosylation strategy based on neighbouring group participation whereby the anomeric stereoselectivity is controlled by a chiral auxiliary at C-2 of the glycosyl donor (Scheme 2.6). The chiral auxiliary is a substituted ethyl moiety that contains a nucleophilic group. Auxiliaries containing either ethoxycarbonyl[158,159] or sulfide[160] substituents have been explored as nucleophiles. As before, activation then loss of the leaving group from the donor produces the oxo-carbenium intermediate. Intramolecular attack by the nucleophilic group on C-2 on either the top or bottom face produces one of two fused decalin systems based on the stereochemistry of the chiral auxiliary. Subsequent substitution by intermolecular nucleophilic attack by the glycosyl acceptor on the anomeric centre of the preferentially formed *trans* decalin system yields the 1,2-*cis* α-glycoside. This novel method, however, does not provide a direct solution to the 1,2-*cis* β-glycosides.

The concept of remote participation has also been addressed to influence glycoside formation.[161,162] Demchenko observed moderate improvements in β-selective glycosidations with *S*-benzoxazolyl mannoside donors employing a remote participating moiety at C-4 (*O*-anisoyl).[163]



Scheme 2.6. *1,2-cis α-glycosylation strategy based on neighbouring group participation.*

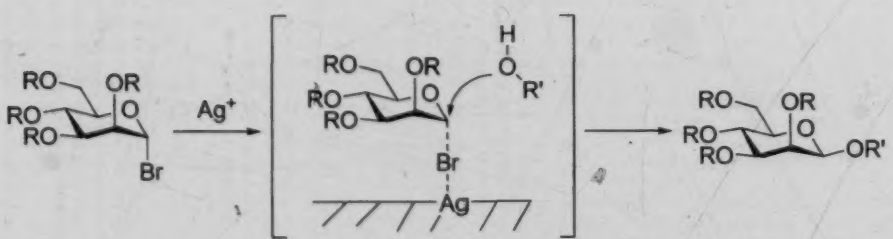
The 1,2-*cis* β-glycosidic linkage found in β-D-mannopyranosides is considered a greater synthetic challenge than the other linkages mentioned. The reliable strategies employed for the synthesis of 1,2-*trans* glycosides and 1,2-*cis* α-glycosides can not be used for the direct and efficient installation of 1,2-*cis* β-glycosidic linkages, including β-D-mannopyranosides. A great number of ingenious approaches to this long-standing problem have been described in the literature; some of the more important strategies will be discussed in section 2.1.3.[164]

2.1.3 Literature methods for the synthesis of β-D-mannopyranosides.

2.1.3.1 Activation of mannosyl halides with insoluble promoters.

An early approach to β-D-mannopyranosides involved the use of an insoluble promoter to activate a glycosyl halide (bromide or chloride) (Scheme 2.7). The first

reported case examined various derivatives of α-D-mannosyl bromides under Königs-Knorr conditions with silver oxide.[165] Improvements in selectivity with less reactive secondary alcohols were realized when silver silicate was used as the insoluble promoter.[166] Preferential formation of the desired β-D-*manno*-configuration can be controlled by using non-participating substituents at C-2 and ensuring that an S_N2-like reaction proceeds on the α-mannosyl halide. Formation of any oxo-carbenium ion (S_N1 reaction) would lead to α-glycosylation due to the anomeric effect.[167] The hypothesis is that the catalyst activates the leaving group at the solution-solid interface. Once the glycosyl donor is activated the solid-support catalyst blocks the α-face and the acceptor is directed to form the β-mannoside. With simple alcohols this method gives good selectivity but fails with less reactive pyranose acceptors. This method has been used to make a β-D-mannopyranosyl-(1→2)-β-D-mannopyranoside disaccharide with moderate stereoselectivity.[168,169]



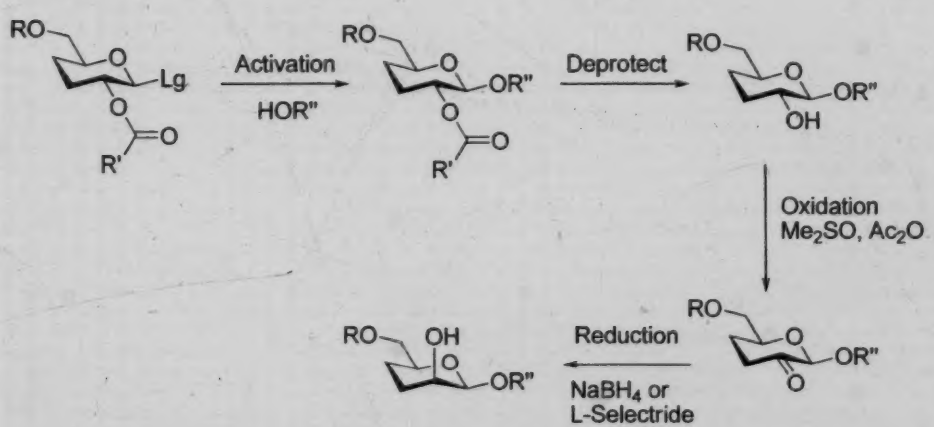
Scheme 2.7. *Formation of β-D-mannopyranosides using the insoluble promoter strategy.*

2.1.3.2 Formation of β-D-mannopyranosides *via* inversion at C-2.

An alternative approach to the use of an insoluble promoter was desired that would provide higher β-stereoselectivity. Synthesis of the 1,2-*trans*-glycoside is much

easier than the 1,2-*cis* isomer. Thus, initial formation of the 1,2-*trans* β-glucoside employing neighbouring group participation followed by inversion of stereochemistry at C-2 could allow access to β-D-mannopyranosides. The key to this strategy is the inversion step and two methods have been utilized: (1) an oxidation-reduction sequence and (2) S_N2 displacement of a C-2 leaving group.

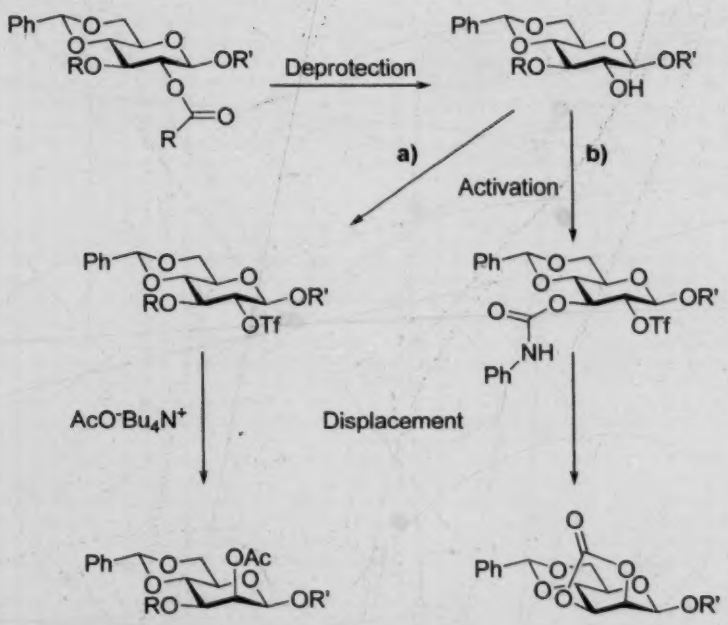
The oxidation-reduction method for the synthesis of β-mannopyranosides was first introduced by Lindberg.[170] Following stereoselective β-glucosylation then deprotection of the 2-*O*-acyl group, oxidation of the C-2 hydroxyl to the ulose is typically carried out under Albright-Goldman[171] conditions with acetic anhydride in Me_2SO (Scheme 2.8). The reductions are carried out using sodium borohydride, or, for more difficult reductions, L-Selectride® is used for improved stereoselectivity.[172,173] Fraser-Reid following the same oxidation-reduction strategy has employed an *n*-pentenyl orthoester based approach to prepare the (1→2)-linked oligomannans of *C. albicans*.



Scheme 2.8. *Formation of β-D-mannopyranosides via the oxidation-reduction method.*

Inversion of the C-2 stereochemistry has also been accomplished using nucleophilic displacement (Scheme 2.9). This strategy commonly relies upon directly converting an alcohol to a sulfonate ester; creating a highly reactive leaving group at the centre to be inverted. Subsequent intermolecular reaction with a strong nucleophile results in S_N2 displacement of the leaving group and inversion of the C-2 configuration (Scheme 2.9a). Typically, triflates are used as leaving groups (though other sulfonates have been used) in combination with a tetrabutylammonium salt of the nucleophile.[174] Fürstner made significant improvements to the displacement by introduction of ultrasound.[175] Intermolecular displacements can be complicated by side products including elimination and ring contraction. Also, the required axial attack of the charged nucleophile can be hampered by unfavourable electronic effects.

An intramolecular version of the displacement strategy was introduced by Kunz and Günther (Scheme 2.9b).[176] In this situation, glucosyl donors were protected with a carbamate group at C-3. Following β-glucosylation and removal of the 2-*O*-acyl group the exposed hydroxyl is converted to a reactive triflate leaving group. The carbonyl oxygen of the carbamate is capable of attacking at C-2 on the top face to displace the triflate resulting in inversion. The expected 2,3-cyclic carbonates are obtained in high yields. Attack by the internal nucleophile is favoured; however, the extra protecting group manipulations required to install the carbamate and the 4,6-*O*-benzylidene acetal reduces the generality of this approach.



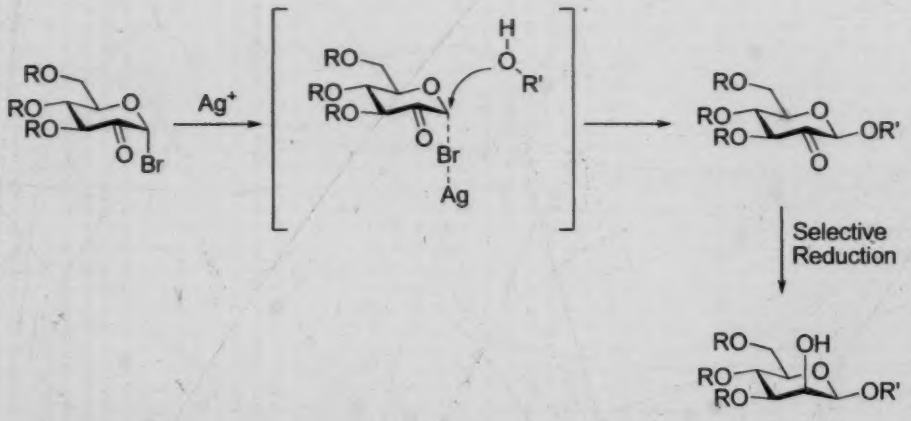
Scheme 2.9. *Formation of β-D-mannopyranosides via displacement a) inter-molecular and b) intra-molecular.*

In the previous inversion methods it was necessary to uniquely expose the C-2 hydroxyl group following glycosylation. Thus, the strategy is complicated by the need for orthogonal protection of the C-2 hydroxyl. To avoid this, Danishefsky has applied the glycal strategy for the synthesis of the β-D-mannopyranosides.[177] The added advantage of this approach is that glycosidation of the 1,2-anhydro donor produces a unique free C-2 hydroxyl in the β-glucopyranoside, which is then available for inversion.

2.1.3.3 The ulosyl bromide method.

In the previous section the synthesis of β-D-mannopyranosides was achieved indirectly by inversion of configuration at C-2 following highly selective β-glucosylation. In the oxidation-reduction sequence a key intermediate is the β-D-glycosid-2-ulose which is then reduced to give the desired β-D-mannopyranosides. Lichtenthaler[178] has

developed an alternative approach to β-D-mannopyranosides from β-D-glycosid-2-uloses via direct glycosidation of 2-oxoglycosyl (ulosyl) bromides (Scheme 2.10). This method avoids the deprotection and oxidation steps required following β-glucosylation. The glycosylation method employed with ulosyl bromides is a variant of the Königs-Knorr conditions where a heterogeneous catalyst activates the bromide and directs attack of the nucleophile to the β-face by blocking the α-face. Although this method proceeds in high stereoselectivity for both the glycosylation and reduction to provide the desired β-D-mannopyranoside, the ulosyl bromide is unstable for storage and must be used immediately following preparation. Bundle and Nitz used this method for the synthesis of di- to hexasaccharide 1,2-linked β-D-mannopyranoside oligomers.[179,180]



Scheme 2.10. *The ulosyl bromide approach for the formation of β-D-mannopyranosides.*

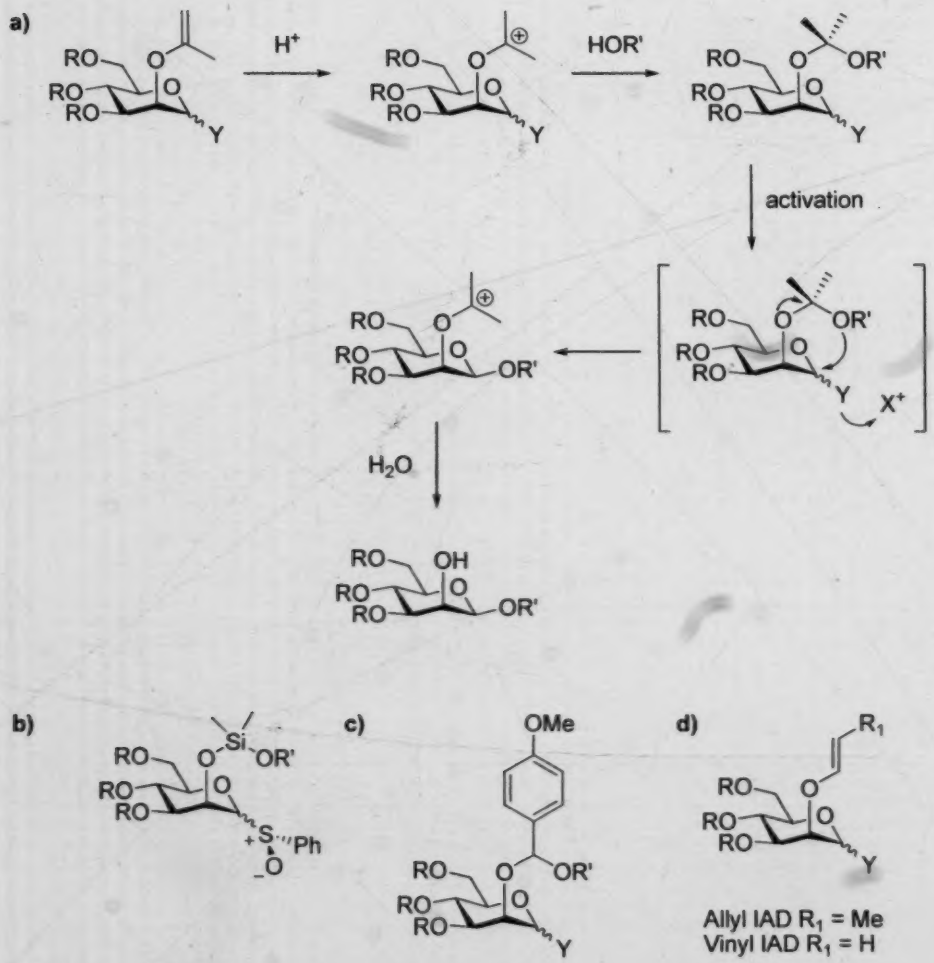
2.1.3.4 Intramolecular aglycone delivery.

A key disadvantage of the strategies described in the previous sections is that none produce β-D-mannopyranosides with complete stereocontrol. Stereospecific

formation of the β-D-mannopyranosidic linkage was addressed by an alternate approach termed intramolecular aglycone delivery (IAD) (Scheme 2.11). The general strategy of IAD involves two critical steps; (1) covalent attachment of the aglycone to *O*-2 of the donor mannopyranose and (2) activation of the donor resulting in concurrent loss of the leaving group and nucleophilic transfer of the tethered aglycone to the anomeric center. The first IAD substrate introduced by Barresi and Hindsgaul was a mixed acetal formed by tethering an acceptor molecule to the thio-mannoside donor through a 2-propenyl ether (Scheme 2.11a).[181-183] Activation of the thioglycoside under standard conditions results in loss of the leaving group then stereospecific, intramolecular transfer of the acceptor to the anomeric center. The reaction gave acceptable yields for disaccharides.

Other research groups have extended the methodology by introducing different tethering chemistry's (Scheme 2.11b-d). Stork and Kim,[184] used temporary silicon connection of acceptors to sulfoxide donors for the stereocontrolled synthesis of disaccharides. While, Ogawa and Ito,[185] introduced mixed acetals of *p*-methoxybenzylidene as the stereocontrolling element in a [2+2]-glycosylation for the preparation of a tetrasaccharide component of the core pentasaccharide and moreover, adapted their method for synthesis on a polymer support.[186] Mixed acetals prepared from allyl and vinyl ethers have also been introduced by Fairbanks, extending the work of Hindsgaul.[187,188] Recently, an IAD method based on 2-*O*-naphthylmethyl ethers was introduced to mediate 1,2-*cis* glycosylations.[189]

Though all of these methods yield the desired β-D-mannopyranosides in stereospecific fashion, additional steps are required to form the tethered intermediates prior to glycosidation.

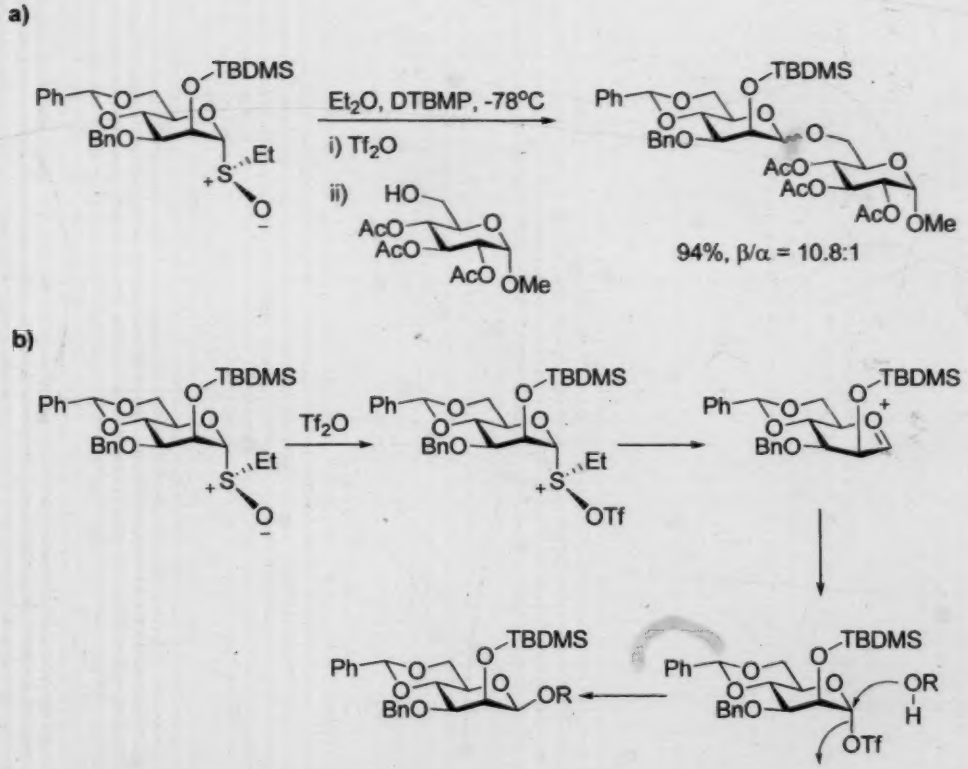


Scheme 2.11. *Synthesis of β-D-mannopyranosides by Intramolecular Aglycone Delivery. a) Hindsgaul IAD b) Stork IAD based on temporary silicon connection c) Ogawa IAD based on mixed acetals of p-methoxybenzylidene d) Fairbanks IAD based on allyl and vinyl ethers.*

2.1.3.5 Displacement with inversion of α-mannosyl triflate donors.

The methods described previously for the preparation of β-D-mannopyranosides either required additional transforming steps following β-glycosylation, lacked high β-selectivity or had limited substrate scope. The most direct method for the synthesis of β-D-mannopyranosidic linkages was developed by Crich and Sun (Scheme 2.12).[190,191]

There strategy involved reaction of a sulfoxide donor, obtained from *m*CPBA-oxidation of the corresponding thioglycoside, with triflic anhydride and a glycosyl acceptor. The 1,2-*cis* β-stereoselectivity was found to be significantly dependent on the mode of addition; it was necessary to react the sulfoxide first with triflic anhydride followed by the glycosyl acceptor. The reaction mechanism is believed to proceed through activation of the leaving group by reaction with triflic anhydride followed by loss of the leaving group leading to formation of the α-mannosyl triflate (Scheme 2.12b). Subsequent addition of the acceptor results in an S_N2-like process with formation of the β-D-mannopyranoside.[192] Enhanced β-selectivity was observed by changing the solvent from diethyl ether to CH_2Cl_2 and reducing the size of the O-2 protecting group on the donor.[193-195] Further improvements on the methodology were made with the observation that the thiophilic reagent benzenesulfenyl triflate (PhSOTf) could readily convert simple thioglycoside donors to glycosyl triflates at low temperature.[196] This advancement was employed for the solid-phase synthesis of β-mannosides and the direct chemical synthesis of a β-(1→2)-D-mannooctaose.[197,198] Though this method allows for the direct and highly stereoselective formation of the β- mannopyranosidic linkage the current methodology is limited to the use of rigid, 4,6-*O*-benzylidene protected glycosyl donors.[199]



Scheme 2.12. *Synthesis of β-D-mannopyranosidic linkages via displacement with inversion of α-mannosyl triflate donors.*[194]

2.2 Synthesis of methyl β-D-mannopyranosyl-(1→2)-β-D-mannopyranoside and deoxy and *O*-methyl analogues.

2.2.1 General considerations and retrosynthetic analysis.

The structures of complementary mono-deoxy and mono-*O*-methyl analogues of methyl β-D-mannopyranosyl-(1→2)-β-D-mannopyranoside raised two important synthetic challenges concerning stereoselectivity and regioselectivity:

(1) How would the challenging β-*manno*-glycosidic linkage be formed, and

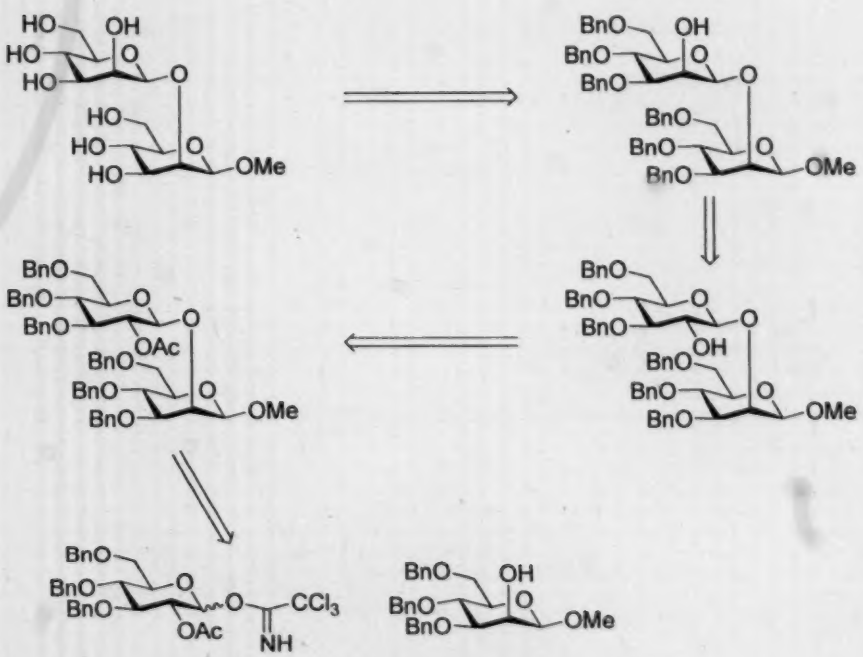
(2) How would each hydroxyl group of the disaccharide be differentiated then subsequently modified.

Considering these issues, two approaches were apparent based on hydroxyl modification either before or following glycosylation. The former strategy, employing singly modified donor or acceptor monosaccharides, was favoured where possible as this would reduce the number of reaction steps performed on the valuable disaccharide intermediates.

Each disaccharide target contains two β-*manno*-glycosidic bonds, an internal linkage between the two sugar residues and an external linkage between the methyl aglycone and anomeric carbon of the reducing-end residue. Arguably, construction of the internal linkage poses the greater synthetic challenge. As discussed previously, the chemical literature describes several approaches for the synthesis of the difficult β-*manno*-glycosidic linkage. The method developed by Crich,[198] involving displacement of reactive α-mannosyl triflate intermediates, represents the most direct route to the (1→2)-β-*manno*-series; however, their chemistry has a strict requirement for use of donors protected with a 4,6-*O*-benzylidene acetal. Thus, substrates with deoxy or *O*-methyl modification at either C-4 or C-6 would not be suitable donors using Crich's α-mannosyl triflate displacement method.

Our general strategy for the synthesis of the (1→2)-β-*manno*-glycosidic linkage is outlined in the retrosynthetic analysis of **1** (Scheme 2.13). The deprotected disaccharide is available from a benzylated methyl β-D-mannopyranosyl-(1→2)-β-D-mannopyranoside intermediate. The β-*manno*-linkage of the non-reducing residue is available from the β-*gluco*-stereochemistry via inversion of the deprotected C-2′ hydroxyl following a

standard two-step oxidation-reduction sequence. The disaccharide can be prepared in both high yield and β-selectivity by glycosylation of a suitably protected acceptor with a 2-*O*-acyl trichloroacetimidate (or thio) glucosyl donor. The donor and acceptor monosaccharides are available in large quantities following known routes from commercially available β-D-glucose penta-acetate and methyl β-D-glucopyranoside, respectively. The latter provided the desired β-stereochemistry of the external glycosidic linkage between the methyl aglycone and anomeric carbon of the reducing-end residue. Importantly, this three-step strategy to the (1→2)-β-*manno*-series, involving highly β-stereoselective glucosylation then inversion of C-2 stereochemistry *via* an oxidation-reduction sequence, enables the use of singly modified donor and acceptor substrates.



Scheme 2.13. *Retrosynthesis of disaccharide 1.*

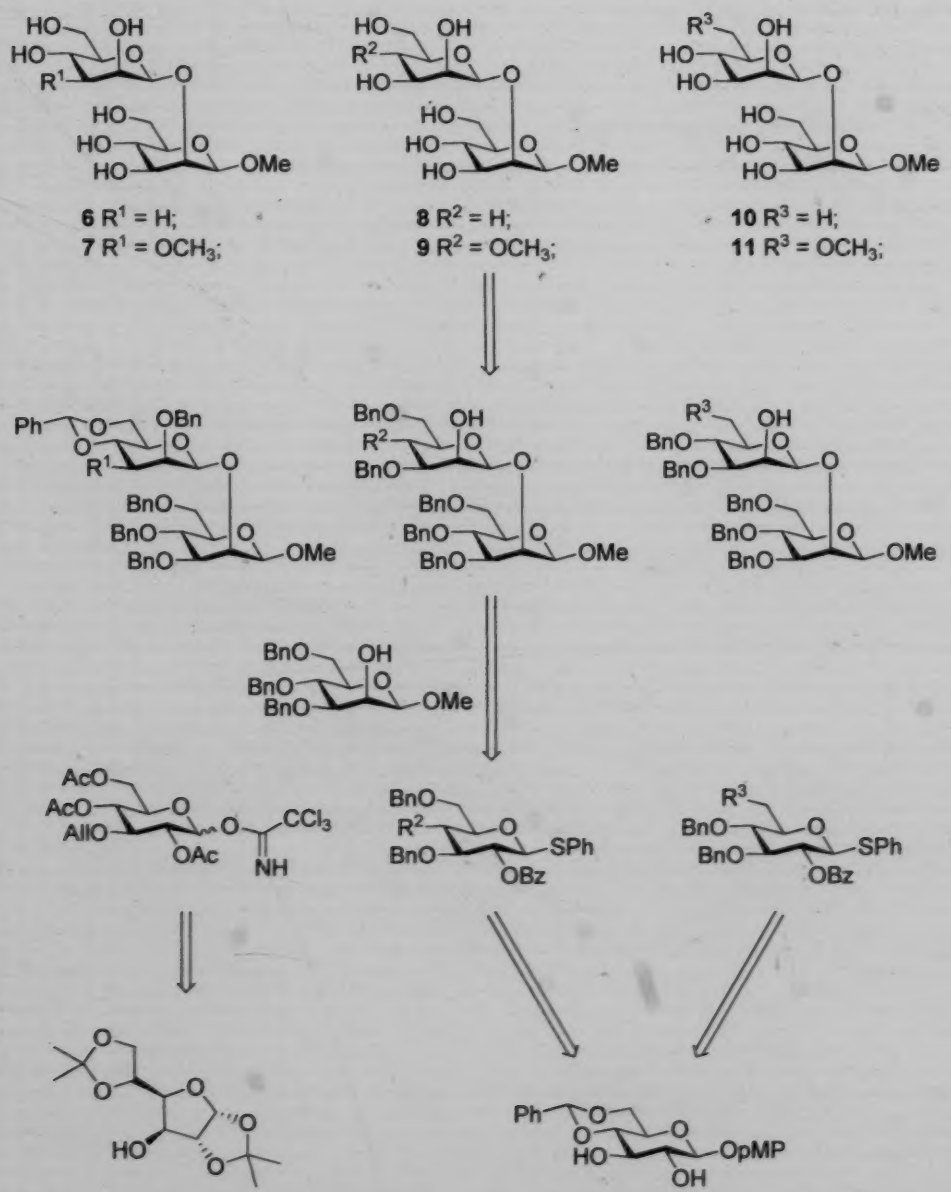
The second major synthetic issue concerned the regioselective single modification of each hydroxyl group of the native disaccharide. Differentiation of each hydroxyl while blocking the remaining is required to avoid over methylation or deoxygenation. The 4,6-*O*-benzylidene acetal gave the dual advantages of protection and access to either the C-4 or C-6 hydroxyl group via regioselective reductive ring opening. Regioselective introduction of other orthogonal protecting groups (i.e. benzoyl, allyl and *para*-methoxy benzyl) was valuable for temporarily blocking selected hydroxyls along the synthetic routes. For all synthetic targets persistent benzyl protection was employed to block hydroxyl groups not selected for isomerization or functional group modification. The benzyl protecting group is stable to most conditions, including methylation and deoxygenation and is easily removed in a final catalytic reduction step.

The series of deoxy and *O*-methyl modified disaccharides, outlined in chapter 1, were divided into two groups; the retrosyntheses of analogues modified on either the non-reducing (**6-11**) or reducing (**12-17**) monosaccharide residue are shown in Schemes 2.14 and 2.15, respectively. Analogues **3-5** were synthesized from intermediates used in the synthesis of the native disaccharide **1**. The analogues were synthesised following a similar route to that of compound **1**, whereby the β-*manno* glycosidic linkage of the non-reducing residue was installed using 2-*O*-acyl *gluco*-donors followed by inversion of stereochemistry at C-2′ and removal of benzyl groups. General retrosynthetic analyses show that the deprotected deoxy and *O*-methyl analogues are available from modified benzylated disaccharide intermediates. The protected disaccharides could be prepared via glycosylation employing a panel of mono-deoxy and mono-*O*-methyl donor or acceptor monosaccharide intermediates coupled with a suitably benzylated acceptor or donor

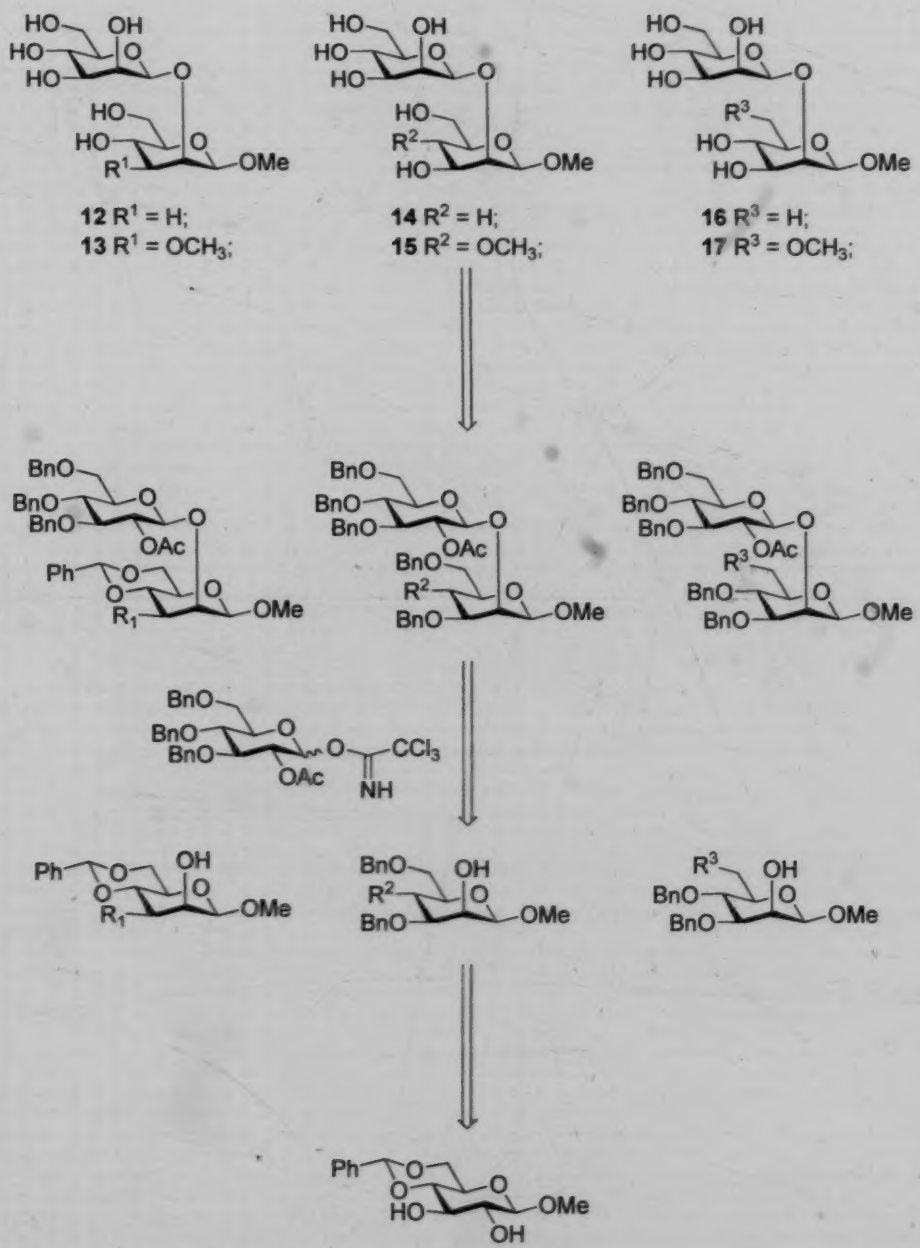
substrate. Methyl 3,4,6-tri-*O*-benzyl β-D-mannopyranoside[183] **20** and 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate[175] **21** were utilized as standard benzylated acceptor and donor substrates, respectively.

Hydroxyl modifications were made on substrates with the *p*-methoxyphenyl[200] temporary anomeric protecting group to avoid possible complications involving activation of sulphur during methylation with methyl iodide or deoxygenation. The thioglucopyranoside donors employed for the synthesis of analogues modified on the non-reducing monosaccharide were readily available from these modified *p*-methoxyphenyl intermediates.

The designation of mono-*O*-methyl analogues as singly modified refers to *O*-methylation in addition to the methyl aglycone.



Scheme 2.14. *Retrosynthesis of analogues (6-11) modified on the non-reducing monosaccharide residue.*



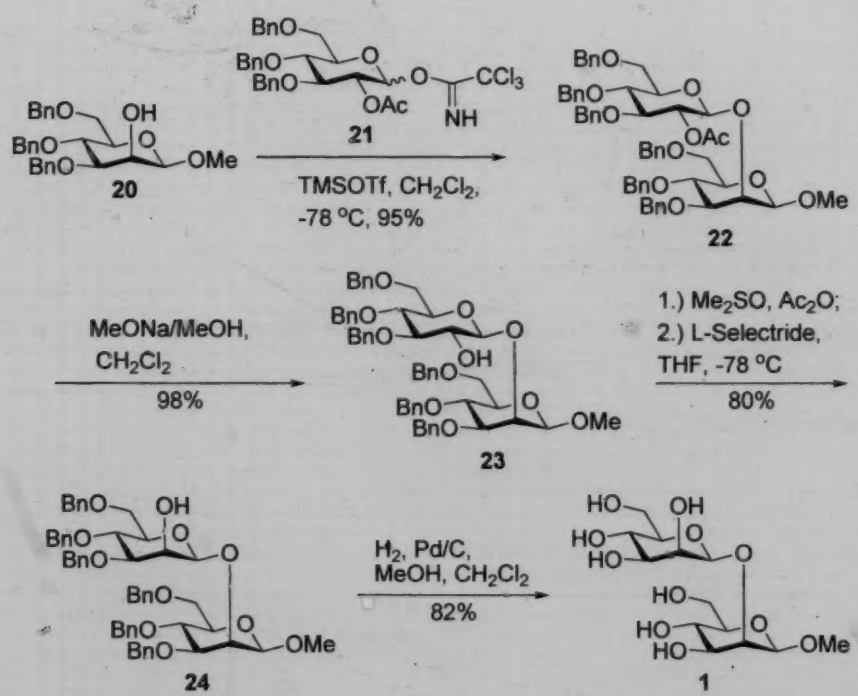
Scheme 2.15. *Retrosynthesis of analogues (12-17) modified on the reducing monosaccharide residue.*

2.2.2 Synthesis of (1→2)-β-D-mannopyranosyl disaccharide and trisaccharide.

The synthesis of disaccharide **1** followed the procedure of Wu and Bundle[201] as outlined in Scheme 2.16. Glycosylation of methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside[183] **20** with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate[175] **21** was performed with TMSOTf activation in CH_2Cl_2 at 0 °C to afford the desired disaccharide **22** in excellent 95% yield. Empirical measurements of carbohydrate one-bond $^1J_{C1,H1}$ heteronuclear coupling constants have shown that values below 165 Hz are indicative of the β-anomer (i.e. the proton attached to the anomeric carbon is bound in the axial orientation).[202] Typically, $^3J_{1,2}$ coupling for β-*gluco* structures is between 8-10 Hz and for β-*manno* <1 Hz. The one-bond $^1J_{C1',H1'}$ heteronuclear coupling constant of the non-reducing monosaccharide residue measured 164.1 Hz, while the $^3J_{1',2'}$ coupling constant measured 9.7 Hz, together indicating the formation of the β-glucopyranosidic linkage. For the remainder of the targets the one-bond $^1J_{C1,H1}$ heteronuclear coupling constants were only measured for the deprotected final compounds to confirm the β-stereochemistry of the glycosidic linkages.

Removal of the acetyl group of **22** under Zemplén transesterification conditions provided **23** (98% yield). The following standard two-step oxidation sequence was used throughout this work for the inversion of β-*gluco* configuration to β-*manno*. Oxidation of **23** with Me_2SO/Ac_2O[171] was followed by reduction with L-Selectride® in THF at -78 °C to give the desired product **24** in 80% yield (exclusively *manno*). Inversion of the C-2′ stereochemistry was followed by noting the change, from starting material to product, of the measured $^3J_{1',2'}$ homo-nuclear coupling constant for the non-reducing monosaccharide

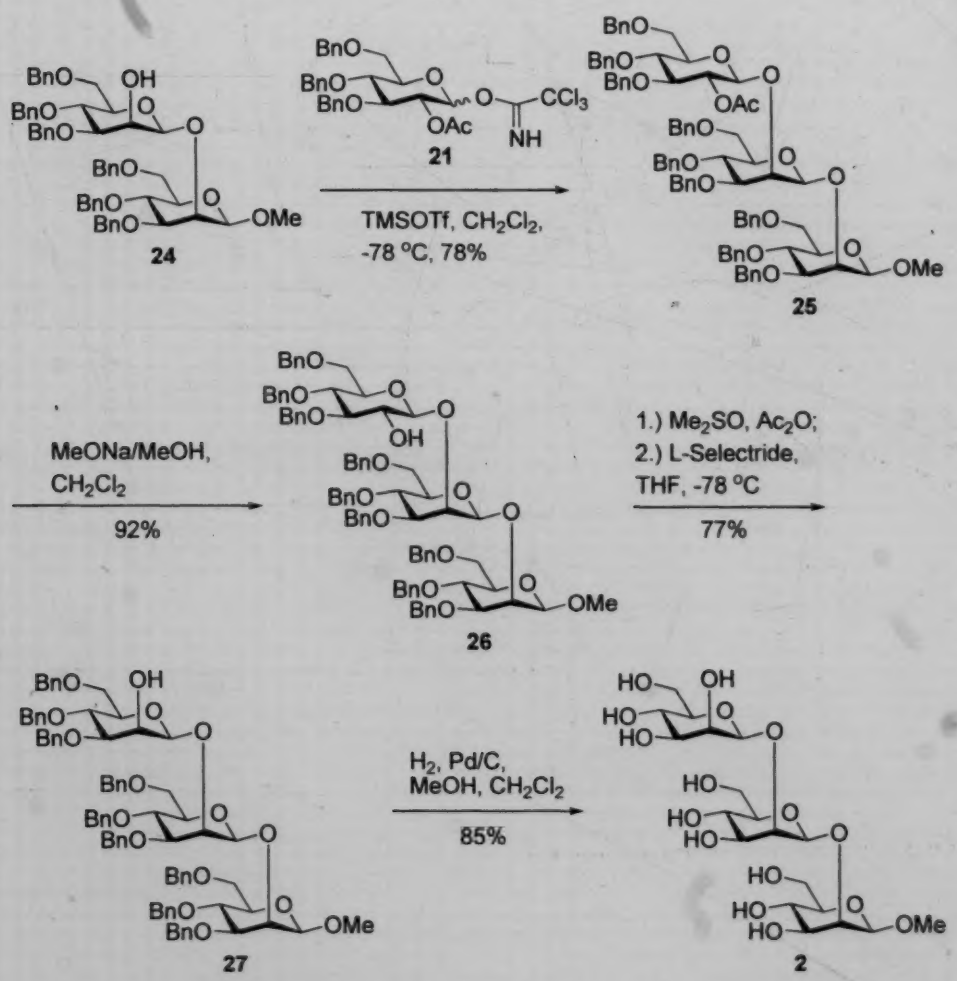
residue. For **23** and **24**, $^{3}J_{1',2'}$ measured 8.1 and 0.7 Hz respectively indicating inversion from the β-*gluco* to the β-*manno* configuration. Global debenzylation under catalytic hydrogenolysis conditions afforded the native disaccharide **1** in 82% yield. The β-mannopyranoside stereochemistry was confirmed by the $^{1}J_{C\text{-}1',H\text{-}1'}$ and $^{1}J_{C\text{-}1,H\text{-}1}$ coupling, 162.7 and 161.2 Hz respectively.



Scheme 2.16. *Synthesis of methyl β-D-mannopyranosyl-(1→2)-β-D-mannopyranoside (1).*

The trisaccharide **2** was synthesized by iteration of the method used to make the disaccharide **1** (Scheme 2.17). Thus, glycosylation of disaccharide **24** with the benzylated trichloroacetimidate donor **21**[175] gave trisaccharide **25**. The $^{3}J_{1'',2''}$ coupling constant (8.2 Hz) confirmed the β-glucosylation. Following the standard deprotection,

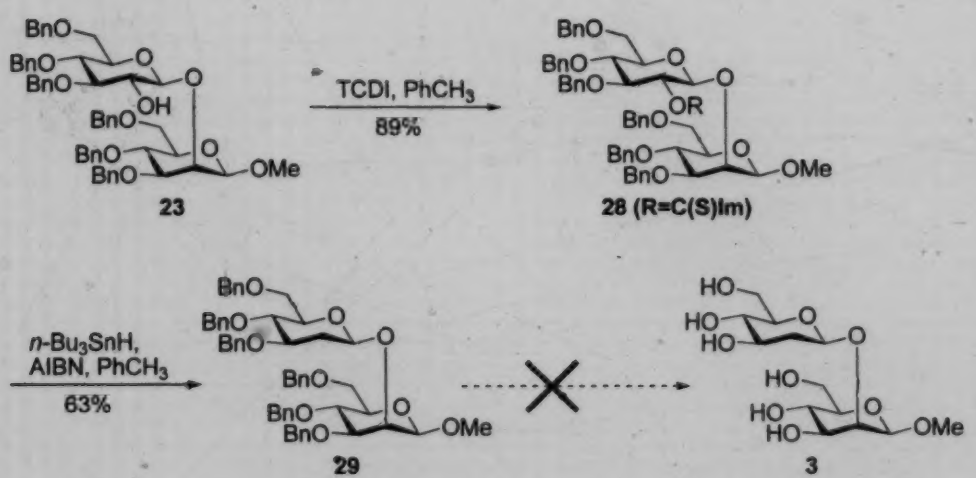
oxidation-reduction sequence and final global deprotection the native trisaccharide **2** was obtained in 60% overall yield (four steps). The $^{1}J_{C\text{-}1'',H\text{-}1''}$, $^{1}J_{C\text{-}1',H\text{-}1'}$ and $^{1}J_{C\text{-}1,H\text{-}1}$ coupling constants measured, 163.0, 159.3, and 159.3 Hz respectively.



Scheme 2.17. *Synthesis of methyl β-D-mannopyranosyl-(1→2)-β-D-mannopyranosyl-(1→2)-β-D-mannopyranoside (2).*

2.2.3 Synthesis of C-2′ modified disaccharide analogues.

The C-2′ modified analogues were readily available from several intermediates used in the synthesis of the native disaccharide **1**. Reaction of disaccharide **23** with thiocarbonyl diimidazole in refluxing toluene gave compound **28** (Scheme 2.18). The Barton-McCombie[203] substrate was then reacted with tributyltin hydride in the presence of AIBN to yield the 2′-deoxy disaccharide **29** in 63% yield. Attempts to purify the 2′-deoxy disaccharide **3** following hydrogenolysis (PdOH) of **29** were unsuccessful. The single polar product obtained from hydrogenation was found to decompose forming two additional spots by TLC following filtration during work-up. The synthesis of **3** was not pursued further due to its instability.

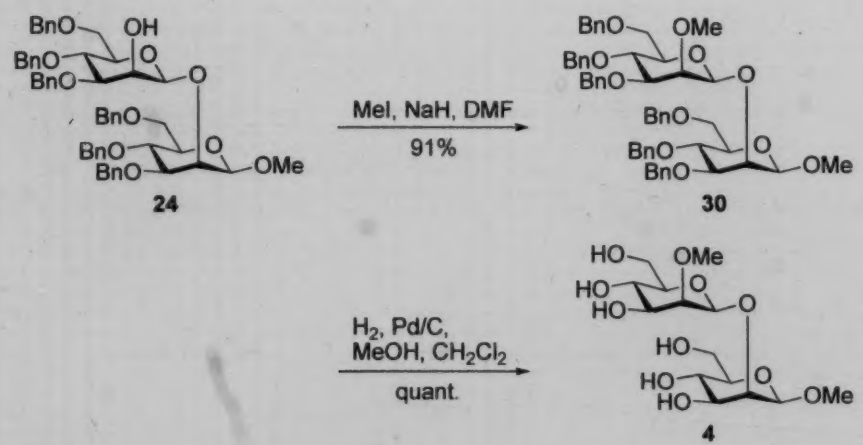


Scheme 2.18. *Attempted synthesis of methyl 2′-deoxy analogue (3).*

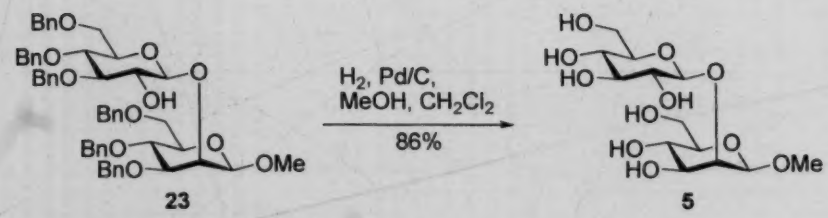
Methylation of **24** with methyl iodide using sodium hydride in DMF gave **30** (91% yield) (Scheme 2.19). The disaccharide was then fully deprotected under catalytic hydrogenation conditions to give the 2′-*O*-methyl disaccharide **4** in quantitative yield.

Analysis of the HMBC spectra showed a correlation between H-2′ (3.83 ppm) and methyl carbon (62.6 ppm) indicating C-2′ hydroxyl methylation.

Global debenzylation of disaccharide **23** under catalytic hydrogenation conditions readily afforded **5** in 86% yield (Scheme 2.20). The $^{1}J_{C\text{-}1,H\text{-}1}$ coupling constants for the non-reducing and reducing end monosaccharide residues measured 165.0 and 160.2 Hz respectively, confirming the β-stereochemistry of both linkages. The three-bond $^{3}J_{1',2'}$ coupling constant of the non-reducing residue measured 7.9 Hz confirming the β-*gluco* configuration.



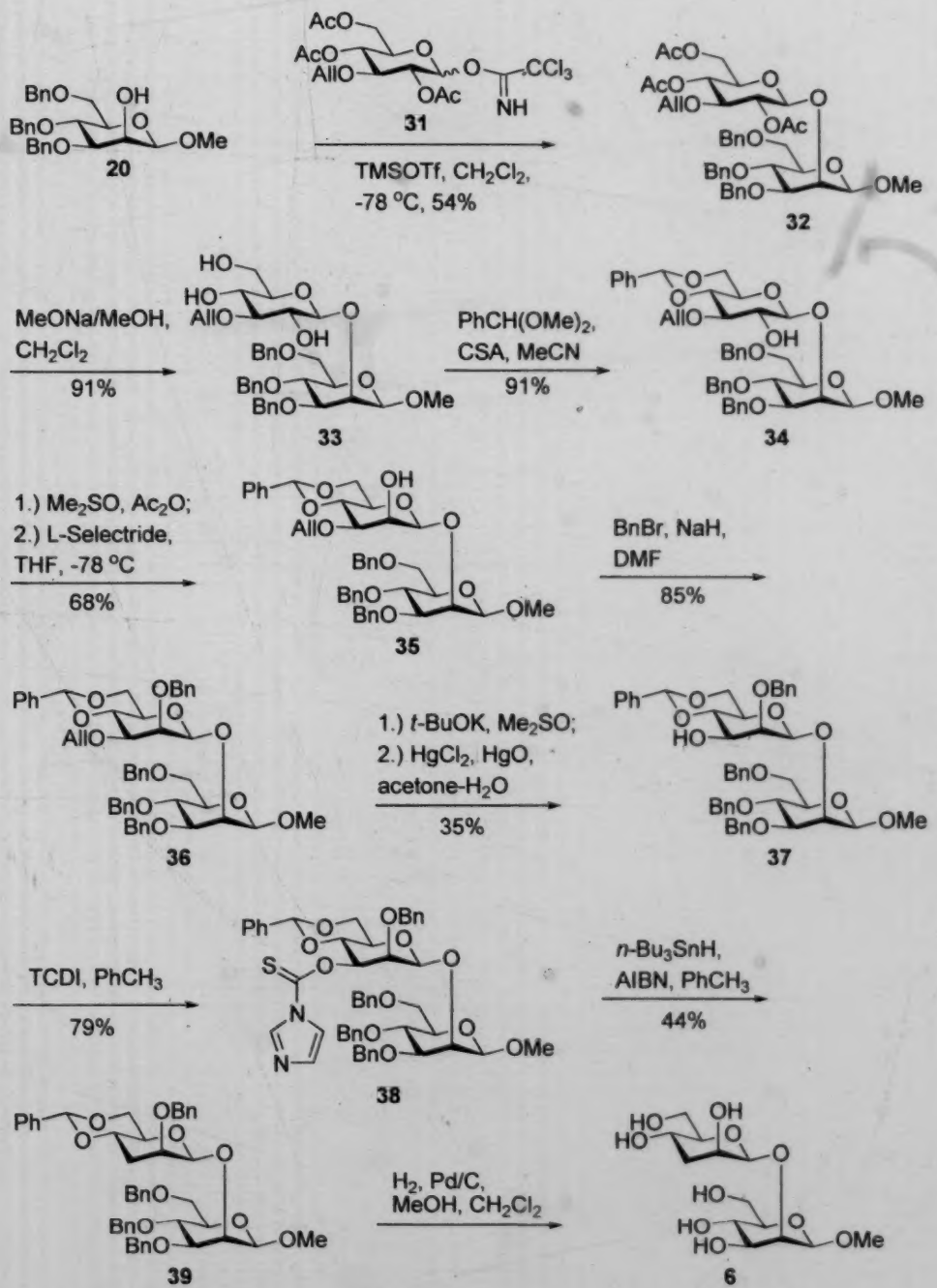
Scheme 2.19. *Synthesis of methyl 2'-O-methyl analogue (4).*



Scheme 2.20. *Synthesis of methyl 2'-gluco analogue (5).*

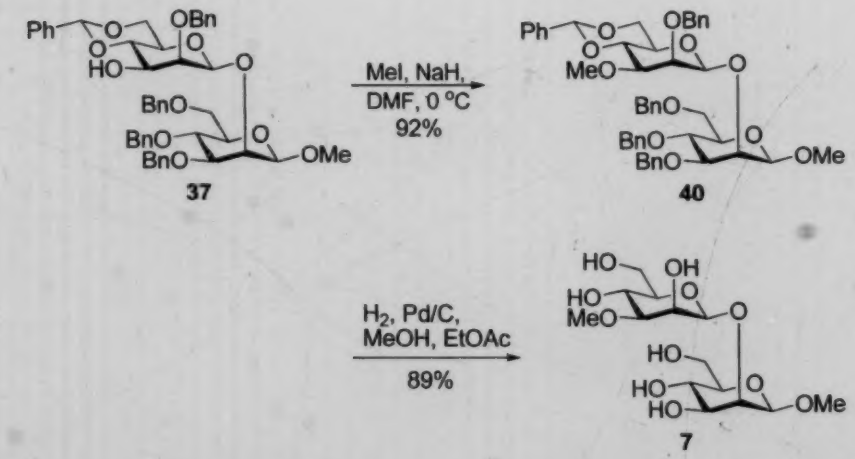
2.2.4 Synthesis of C-3′ modified disaccharide analogues.

The 3′-deoxy and 3′-*O*-methyl analogues, **6** and **7,** were prepared by modification of a common advanced disaccharide intermediate **37**. The common benzylated mannose acceptor **20** was reacted with the known 2,4,6-tri-*O*-acetyl-3-*O*-allyl-β-D-glucopyranosyl trichloroacetimidate[204] **31** using catalytic TMSOTf to form disaccharide **32** (54% yield) (Scheme 2.21). The $^{3}J_{1'',2''}$ coupling constant (8.1 Hz) confirmed the β-glucosylation. Removal of the acetates under Zemplén transesterification conditions followed by installation of the 4,6-*O*-benzylidene acetal gave **34** in 83% yield over two-steps. The standard two-step oxidation-reduction sequence was used to invert the C-2′ *gluco* configuration of **34** to give the *manno* configuration of **35**. The $^{3}J_{1'',2''}$ coupling constant measured 0.9 Hz indicating successful inversion of stereochemistry. Benzylation of the free hydroxyl group followed by allyl removal afforded the desired intermediate **37** (35% yield). Reaction of **37** with thiocarbonyl diimidazole in toluene at reflux gave the Barton-McCombie substrate **38** (79% yield), which was then treated with tributyltin hydride in the presence of AIBN to give **39** (44% yield). The deprotected 3′-deoxy analogue **6** was prepared under catalytic hydrogenation conditions. The reaction conditions and yields were not optimized.



Scheme 2.21. *Synthesis of methyl 3'-deoxy analogue (6).*

Methylation of the free hydroxyl group of **37** using methyl iodide and sodium hydride gave disaccharide **38** (Scheme 2.22). Subsequent hydrogenolysis provided the desired 3′-*O*-methyl target analogue **7**.

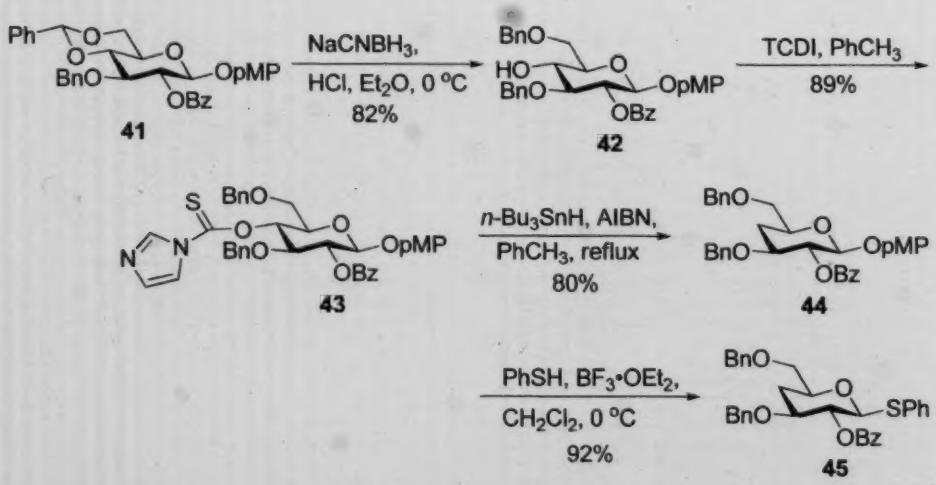


Scheme 2.22. *Synthesis of methyl 3′-O-methyl analogue (7).*

2.2.5 Synthesis of C-4′ modified disaccharide analogues.

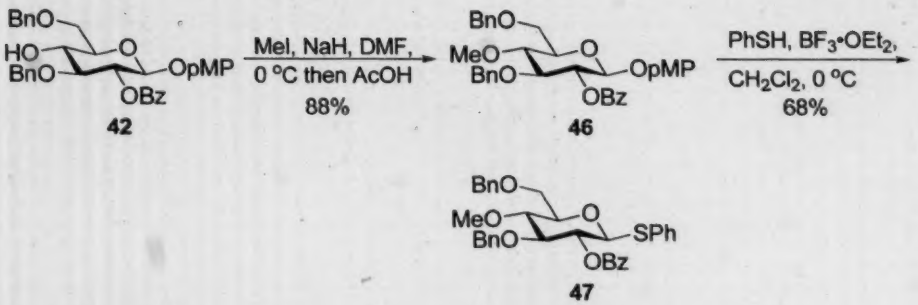
The known *p*-methoxyphenyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside[205] **41** was a suitable orthogonally protected starting material for the preparation of both the 4′-deoxy and 4′-*O*-methyl thioglucoside donors. Reductive opening of the benzylidene acetal under anhydrous conditions with sodium cyanoborohydride and HCl in ether[206] gave the desired alcohol **42** in 82% yield (Scheme 2.23). The regioselectivity was confirmed by subsequent reaction of **42** with thiocarbonyl diimidazole in toluene at reflux to give **43** (89% yield) in which the chemical shift of H-4 moved from 3.88 to 6.07 ppm. The Barton-McCombie substrate was then reacted with tributyltin hydride in the presence of AIBN to yield the 4-deoxy

substrate **44** in 80% yield. Activation of **44** using borontrifluoride-diethyl etherate and subsequent reaction with thiophenol gave the phenyl 4-deoxy thioglucopyranoside donor **45** in 92% yield.



Scheme 2.23. *Synthesis of 4'-deoxy thioglucoside donor (45).*

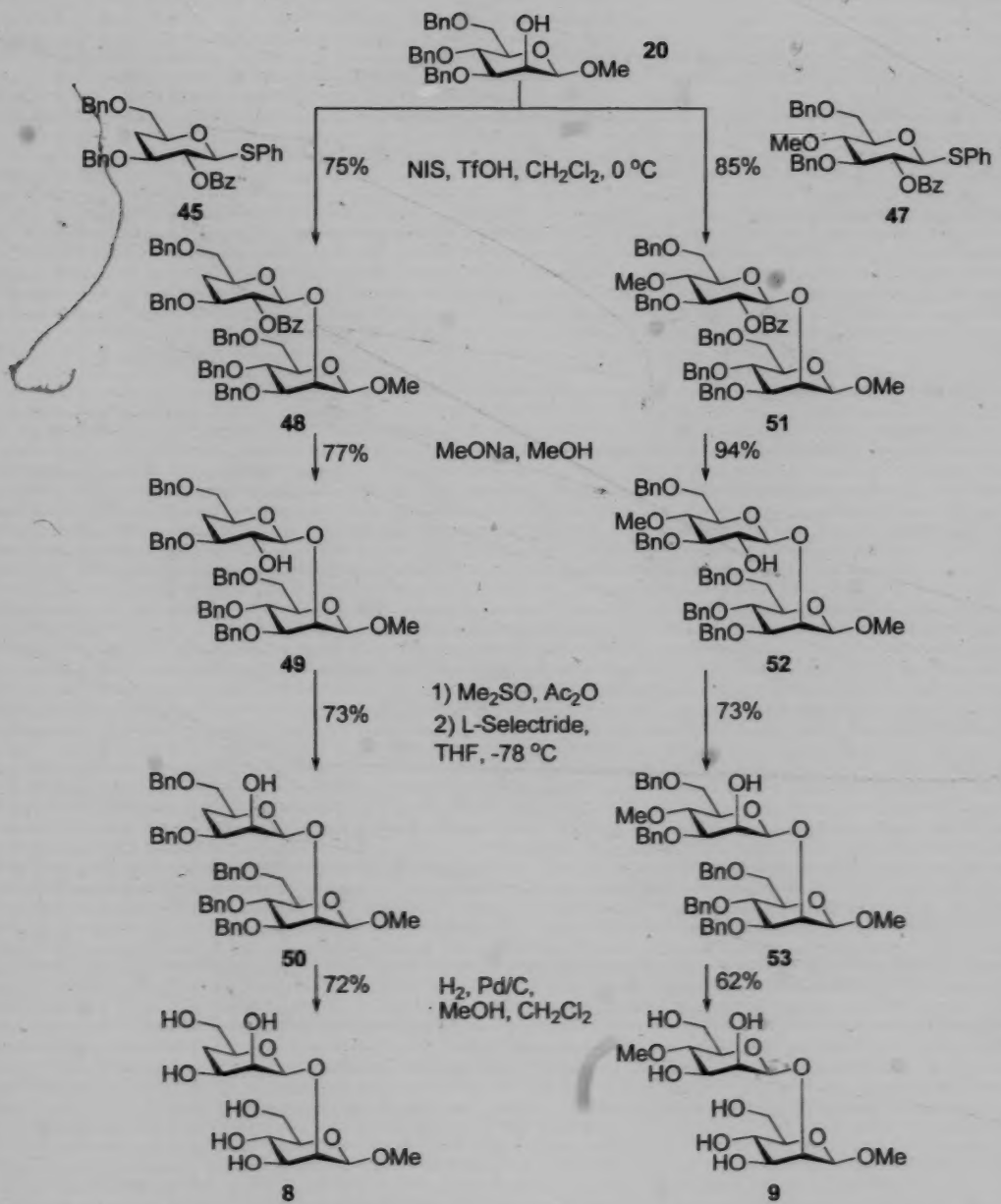
Alcohol **42**, an intermediate used in the preparation of the 4-deoxy thioglucopyranoside donor **45**, was reacted with methyl iodide in the presence of sodium hydride in DMF to give **46** (88%) (Scheme 2.24). Neutralization with acetic acid prior to work-up assured the integrity of the 2-*O*-benzoyl group. The thiophenyl group was then installed by reaction with thiophenol and boron trifluoride-diethyl etherate to give the desired 4-*O*-methyl thioglucoside donor **47** in acceptable isolated yield (68%).



Scheme 2.24. *Synthesis of 4'-O-methyl thioglucoside donor (47).*

The 4-deoxy **45** and 4-*O*-methyl **47** thioglucoside donors were in turn reacted with acceptor **20** using NIS/AgOTf activation to give disaccharides **48** (75% yield) and **51** (85% yield) respectively (Scheme 2.25). The $^{3}J_{1',2'}$ coupling constants measured 8.0 and 8.1 Hz for **48** and **51**, respectively. The disaccharides were then elaborated to the target structures **8** and **9** following standard procedures described for native disaccharide **1**. Treatment of **48** and **51** under transesterification conditions gave alcohols **49** (77% yield) and **52** (94% yield), which were in turn oxidized then reduced to give **50** and **53** both in 73% yield.

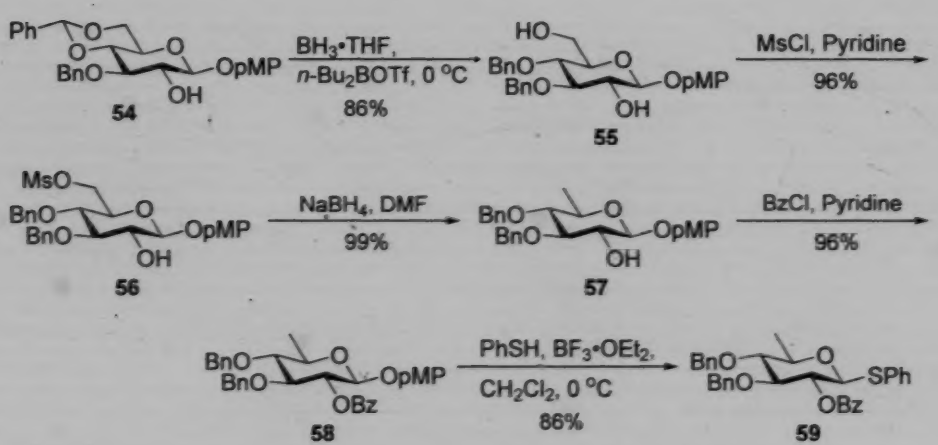
The $^{3}J_{1',2'}$ coupling constants now measured 0.9 Hz for both **50** and **53**, indicating inversion of configuration at C-2'. Global deprotection of the benzyl groups of **50** and **53** under hydrogenolysis gave the desired 4'-deoxy **8** (72%) and 4'-*O*-methyl **9** (quant.) disaccharide analogues respectively. Extensive overlap in the ^{1}H- and ^{13}C-NMR of **8** precluded characterization of the glycosidic bonds. However, for **9** the β-*manno*-stereochemistry was supported by both the $^{1}J_{C1,H1}$ and $^{3}J_{1,2}$ coupling constants.



Scheme 2.25. *Synthesis of 4'-deoxy (**8**) and 4'-O-methyl (**9**) analogues.*

2.2.6 Synthesis of C-6′ modified disaccharide analogues.

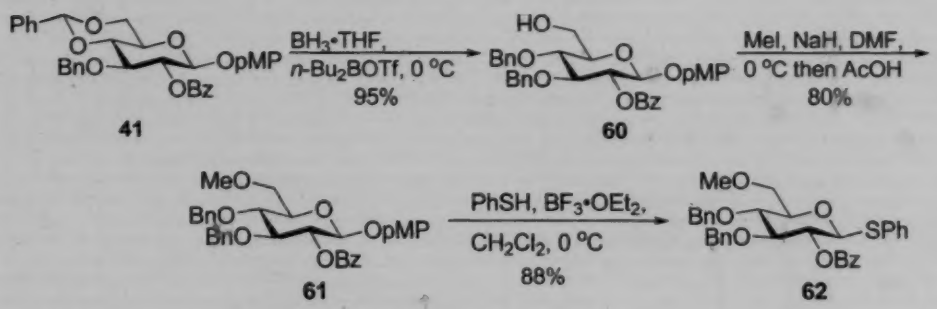
The synthesis of the 6′-deoxy analogue proceeded from the known *p*-methoxyphenyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside[207] **54**. Reductive opening of the benzylidene acetal under anhydrous conditions with BH_3·THF and dibutylboron triflate[208] gave the desired diol **55** in 86% yield (Scheme 2.26). Controlled reaction with methanesulfonyl chloride in pyridine gave **56** in high regioselectivity. The 6-deoxy intermediate **57** was obtained in near quantitative yield by reductive displacement using $NaBH_4$. Benzoylation of **57** followed by Lewis acid mediated reaction with thiophenol gave the 6-deoxy thioglucoside donor **59** in excellent yield. Installation of the 2-*O*-benzoyl group required for neighbouring participation was delayed until after reduction with $NaBH_4$ to avoid reductive loss of the ester group.



Scheme 2.26. *Synthesis of 6′-deoxy thioglucoside donor (59).*

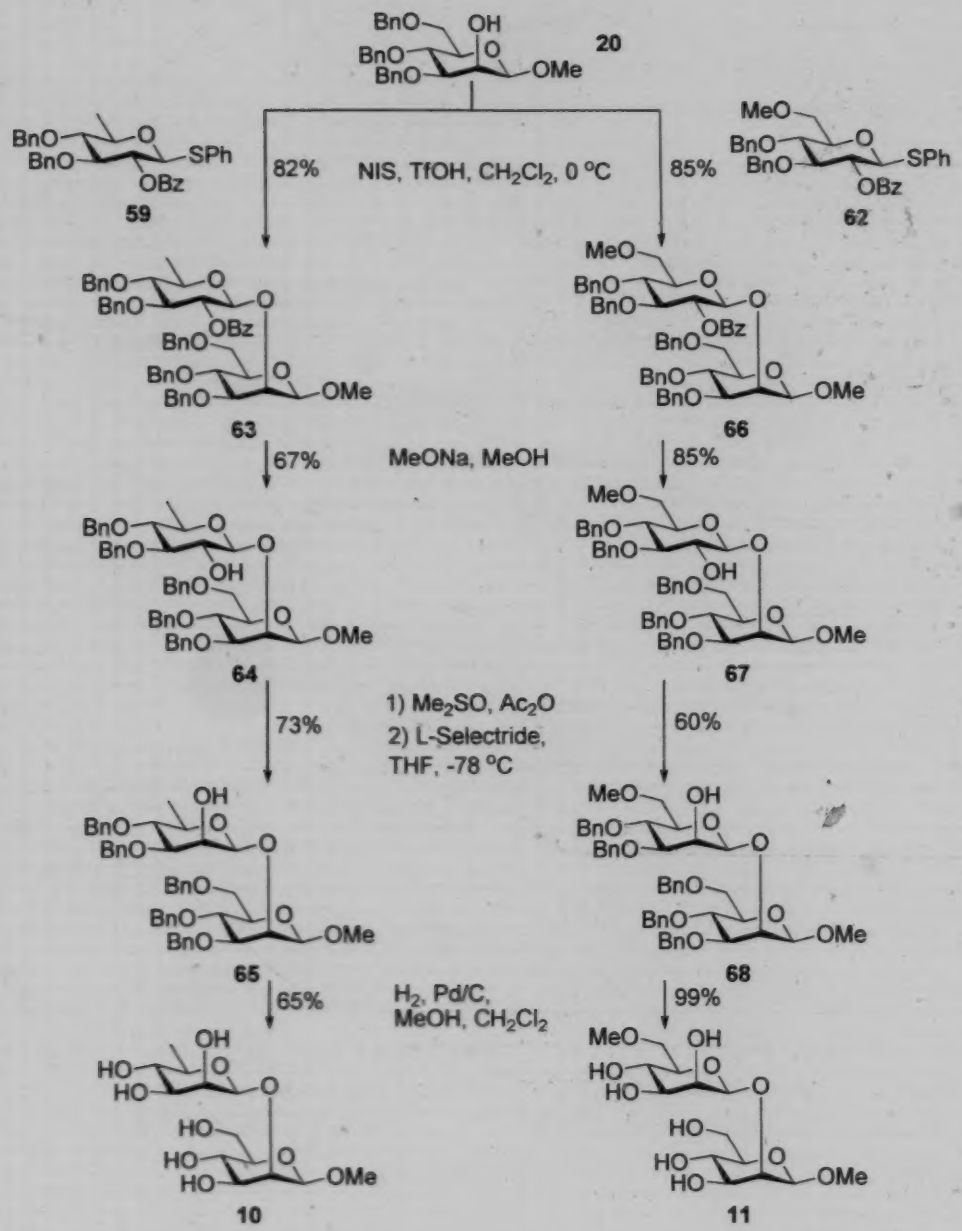
The previously used *p*-methoxyphenyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside **41** was suitable for the synthesis of the 6′-O-methyl

thioglucoside donor. The desired alcohol **60** was obtained in high yield by treatment with BH_3·THF and dibutylboron triflate[208] (Scheme 2.27). Methylation of the primary alcohol using methyl iodide and sodium hydride followed by neutralization with acetic acid gave **61** (80% yield). The 6-*O*-methyl thioglucoside donor **62** was then prepared by reaction with thiophenol and borontrifluoride.



Scheme 2.27. *Synthesis of 6'-O-methyl thioglucoside donor (62).*

Reaction of the 6-deoxy **59** and 6-*O*-methyl **62** thioglucoside donors with benzylated acceptor **20** using NIS/AgOTf activation gave disaccharides **63** (82% yield) and **66** (85% yield), respectively (Scheme 2.28). Treatment of the disaccharides with NaOMe/MeOH followed by the standard oxidation-reduction sequence provided intermediates **65** and **68**. Changes in the observed $^3J_{1',2'}$ coupling constants indicated inversion from the *gluco* to the *manno* configuration. Subsequent hydrogenolysis of **65** and **68** gave the desired 6'-deoxy **10** and 6'-*O*-methyl **11** disaccharide analogues respectively.



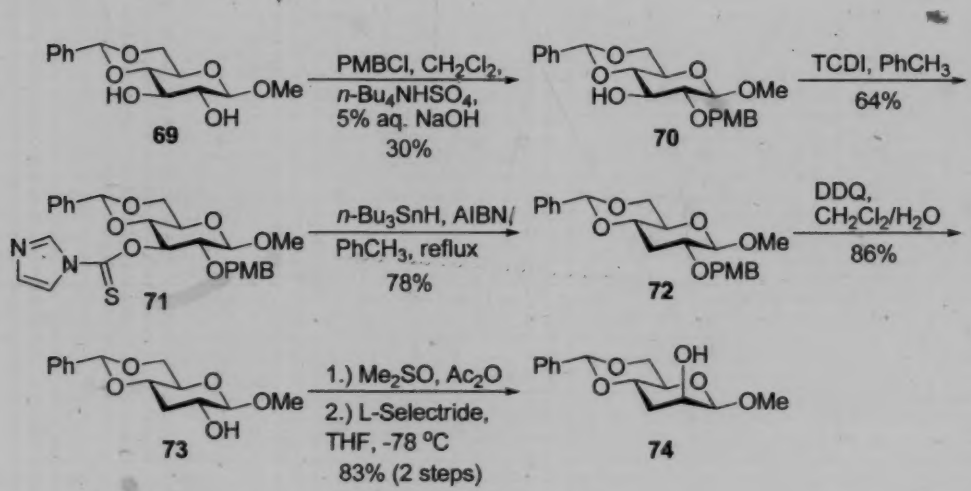
Scheme 2.28. *Synthesis of 6'-deoxy (10) and 6'-O-methyl (11) analogues.*

2.2.7 Synthesis of C-3 modified disaccharide analogues.

Readily available methyl 4,6-*O*-benzylidene-β-D-glucopyranoside **69**[209] was reacted under phase transfer conditions[210] with *p*-methoxybenzyl chloride to give a mixture of 2-*O*- and 3-*O*-monobenzylated regioisomers (Scheme 2.29). Although, the desired 2-*O*-*p*-methoxybenzyl protected intermediate **70** was obtained in low yield (30% yield), it was easily prepared in large scale and purified in sufficient quantities to proceed. Temporary protection of the 2-hydroxyl as the *p*-methoxybenzyl ether allowed for straightforward manipulation of the 3-hydroxyl group. The regiochemistry of **70** was confirmed by subsequent reaction with thiocarbonyl diimidazole in toluene at 80 °C to give **71** in 64% yield; the chemical shift of H-3 moved downfield from 3.82 to 6.11 ppm. The Barton-McCombie substrate **71** was then treated with tributyltin hydride in the presence of AIBN to afford the 3-deoxy intermediate **72** in 78% yield. Removal of the *p*-methoxybenzyl ether with DDQ in wet CH_2Cl_2 gave **73** in 86% yield and exposed the 2-hydroxyl group, which was then inverted from the *gluco* to the *manno* configuration via the standard two step oxidation-reduction sequence.

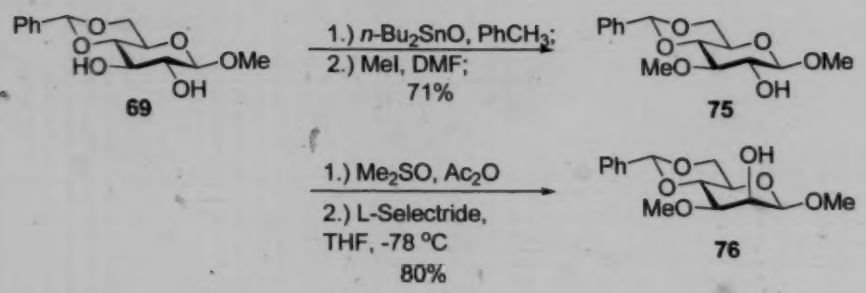
Oxidation of **73** under Albright-Goldman[171] conditions (Me_2SO/Ac_2O) was followed by reduction with L-Selectride® in THF at -78 °C to give **74**. Lichtenthaler et al.[173] investigated the reduction of 2-keto groups with various hydride reagents and found L-Selectride® to reduce the carbonyl of several substrates with high *manno*-selectivity. Conversely, $NaBH_4$ was found to be selective with only fully benzylated substrates and gives poor *manno*- versus *gluco*-selectivity (7:1) with benzylidene containing substrates.[209] In our case oxidation then reduction of the benzylidenated substrate **73** with L-Selectride® gave the desired 3-deoxy mannose acceptor **74** in 83% yield

(exclusively *manno*); the $^3J_{1,2}$ measured 1.3 Hz indicating the β-*manno*-configuration. A small amount of side product (usually 5-10%) was obtained and identified as methyl 4,6-*O*-benzylidene-3-deoxy-2-*O*-methylthiomethyl-β-D-glucopyranoside. It has previously been noted, that the methylthiomethyl ether substituted by-product is formed under Me_2SO/Ac_2O oxidation conditions.[171]



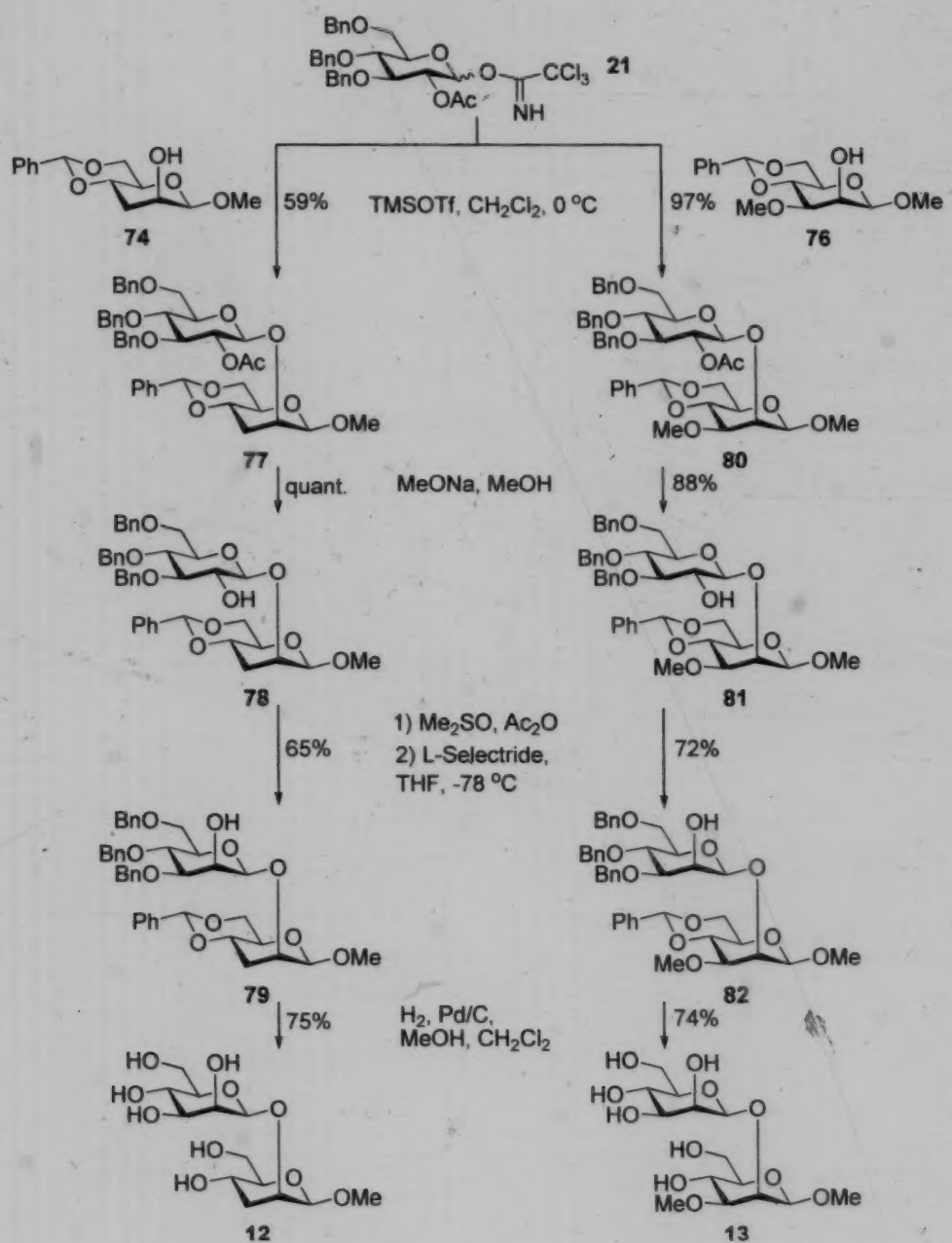
Scheme 2.29. *Synthesis of methyl 3-deoxy-β-D-mannose acceptor (74).*

The 3-*O*-methyl mannose acceptor **76** was prepared starting from direct methylation of methyl 4,6-*O*-benzylidene-β-D-glucopyranoside **69** using stannylidene chemistry (Scheme 2.30). Reaction with dibutyltin oxide in toluene at reflux followed by treatment with methyl iodide in DMF gave **75** in 71% yield. The *gluco*-intermediate **75** was then reacted following the standard oxidation-reduction sequence to afford the 3-*O*-methyl mannose acceptor **76** in 80% yield. The measured $^3J_{1,2}$ coupling constant (1.0 Hz) indicated inversion to the β-*manno*-configuration.



Scheme 2.30. *Synthesis of methyl 3-O-methyl-β-D-mannose acceptor (76).*

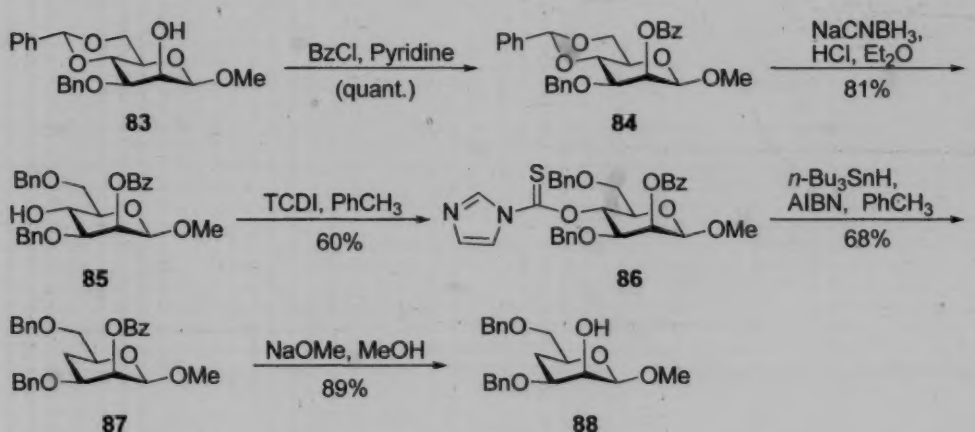
Reaction of the prepared 3-deoxy and 3-*O*-methyl acceptors **74** and **76**, with the known 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate[175] donor **21** gave intermediate disaccharides **77** (59% yield) and **80** (97% yield), respectively (Scheme 2.31). The synthesis of **77** was not optimized. The $^{3}J_{1',2'}$ coupling constants were measured to be 8.0 Hz for both **77** and **80**, indicating β-*gluco* stereochemistry. Transesterification followed by the standard two-step oxidation-reduction sequence converted disaccharides **77** and **80** into intermediates **79** and **82**. Global debenzylation under hydrogenolysis conditions gave the desired 3-deoxy **12** and 3-*O*-methyl **13** target analogues in 75% and 74% yields, respectively. The $^{1}J_{C\text{-}1,H\text{-}1}$ and $^{3}J_{1',2'}$ coupling constants confirmed the β-*manno* configuration for both **12** and **13**.



Scheme 2.31. *Synthesis of 3-deoxy (12) and 3-O-methyl (13) analogues.*

2.2.8 Synthesis of C-4 modified disaccharide analogues.

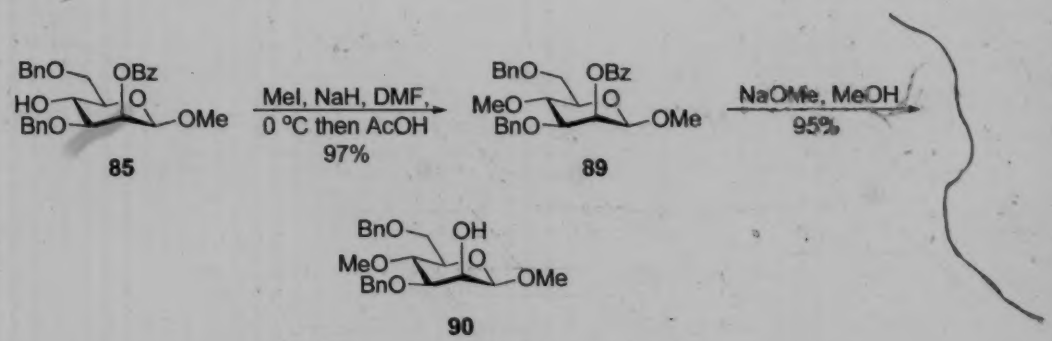
Benzoylation of the known methyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside[209] **83** gave intermediate **84** (Scheme 2.32). Reductive opening of the benzylidene acetal with sodium cyanoborohydride and HCl in ether[206] gave the desired alcohol **85** in 81% yield. The regioselectivity was confirmed by subsequent reaction with thiocarbonyl diimidazole in toluene at reflux to give **86** (60% yield) in which the chemical shift of H-4 moved downfield from 4.04 to 6.24 ppm. Treatment of the Barton-McCombie substrate with tributyltin hydride in the presence of AIBN gave the 4-deoxy substrate **87**. The benzoyl group was removed by transesterification using sodium methoxide in methanol to give the 4-deoxy acceptor **88**.



Scheme 2.32. *Synthesis of methyl 4-deoxy-β-D-mannose acceptor (88).*

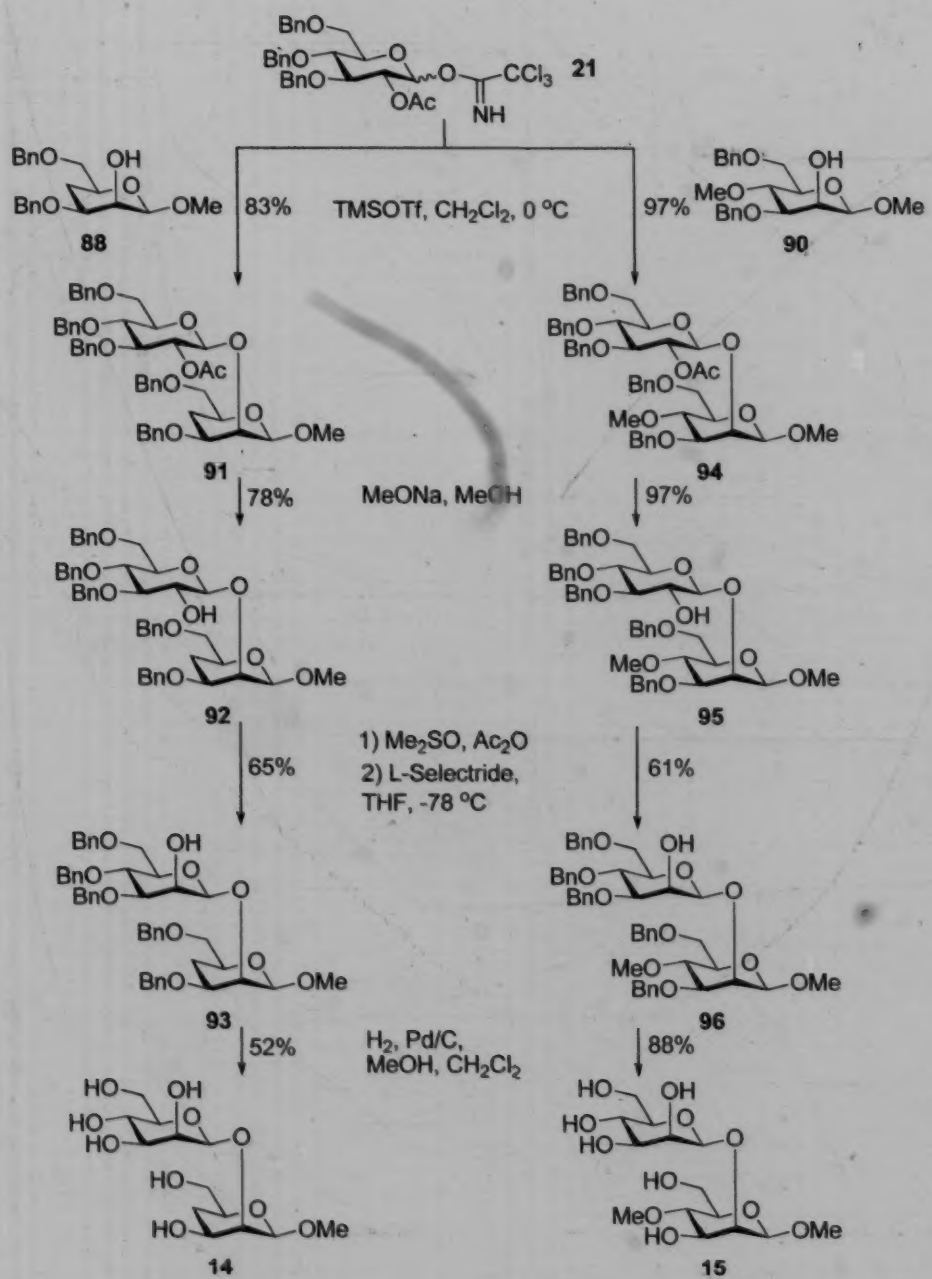
Methylation of intermediate **85** using methyl iodide and sodium hydride followed by quenching with acetic acid gave intermediate **89** (Scheme 2.33). Removal of the

benzoyl group under Zemplén conditions afforded the desired 4-*O*-methyl acceptor **90** in 95% yield.



Scheme 2.33. *Synthesis of methyl 4-O-methyl-β-D-mannose acceptor (**90**).*

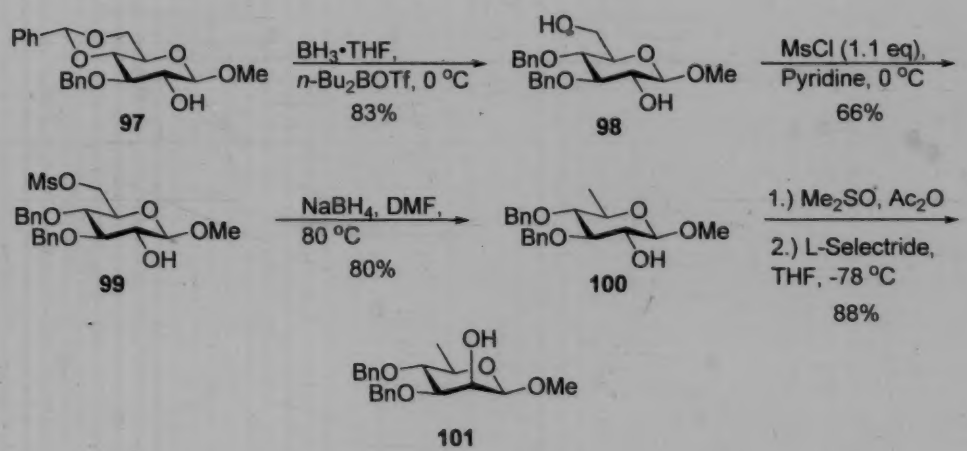
Reaction of the 4-deoxy **88** and 4-*O*-methyl **90** acceptors with benzylated trichloroacetimidate[175] donor **21** under TMSOTf activation gave disaccharides **91** (83% yield) and **94** (97% yield), respectively (Scheme 2.34). The $^{3}J_{1',2'}$ proton coupling constants measured 8.0 and 8.1 Hz for **91** and **94**, respectively. The disaccharides were then treated with NaOMe/MeOH followed by the standard oxidation-reduction sequence to provide intermediates **93** and **96**. The $^{3}J_{1',2'}$ proton coupling constants now measured 0.7 Hz for both **93** and **96**, indicating inversion from the *gluco* to the *manno* configuration. Subsequent hydrogenolysis of **93** and **96** gave the desired 4-deoxy **14** (52% yield) and 4-*O*-methyl **15** (88% yield) disaccharide analogues respectively. The $^{1}J_{C\text{-}1,H\text{-}1}$ and $^{3}J_{1',2'}$ coupling constants confirmed the β-*manno* configuration for both **14** and **15**.



Scheme 2.34. *Synthesis of 4-deoxy (**14**) and 4-O-methyl (**15**) analogues.*

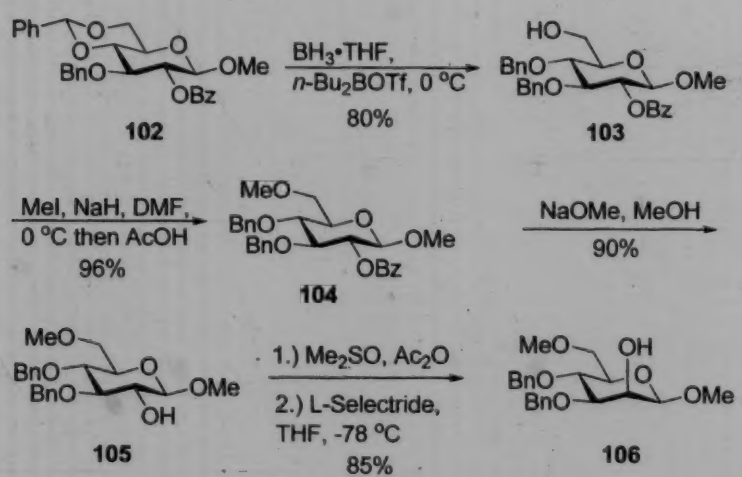
2.2.9 Synthesis of C-6 modified disaccharide analogues.

Reductive benzylidene acetal opening of known methyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside[209] **97** under anhydrous conditions with BH_3·THF and dibutylboron triflate[208] gave diol **98** in 83% yield (Scheme 2.35). Controlled reaction of the C-6 primary hydroxyl with methanesulfonyl chloride in pyridine gave intermediate **99** (66% yield). The downfield shift of the C-6 proton resonances (from 3.89 and 3.75 ppm to 4.47 and 4.37 ppm) confirmed the regioselectivity of both the benzylidene acetal opening and selective sulfonation. The 6-deoxy intermediate **100** was obtained in 80% yield by reductive displacement of the sulfonate using $NaBH_4$. The standard two-step oxidation-reduction sequence was then used to invert the *gluco*-configuration to yield the desired 6-deoxy mannose acceptor **101** (88% yield). Change in the $^3J_{1,2}$ coupling constant (from 7.6 to 1.1 Hz) indicated inversion to the β-*manno*-configuration.



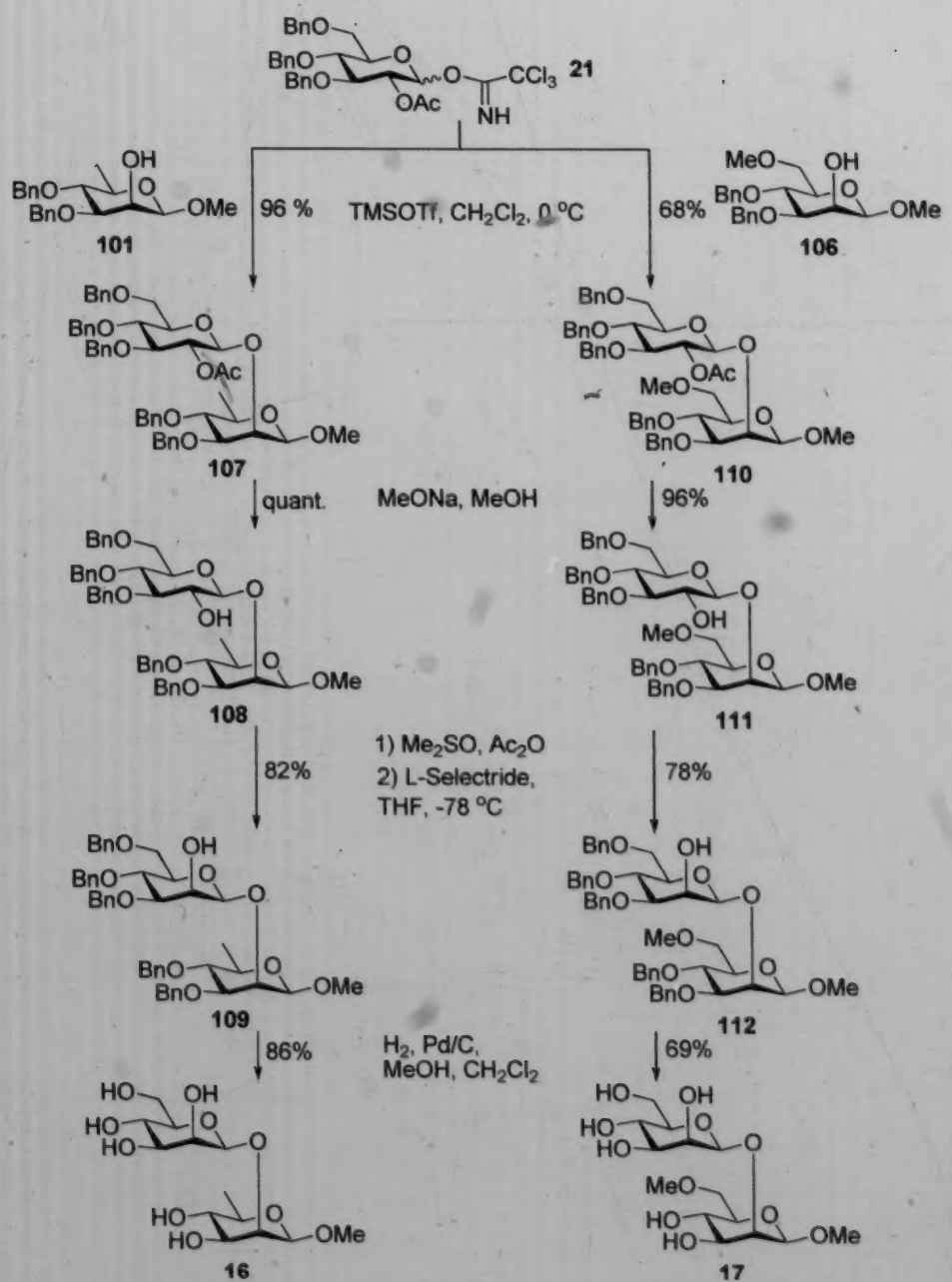
Scheme 2.35. *Synthesis of methyl 6-deoxy-β-D-mannose acceptor (101).*

Reductive benzylidene acetal opening of the known methyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside[211] **102** under anhydrous conditions with BH_3·THF and dibutylboron triflate[208] gave **103** in 80% yield (Scheme 2.36). Methylation of the free C-6 hydroxyl afforded **104** which was than reacted under Zemplén transesterification conditions to give intermediate **105** (86% yield two-steps). Again, the standard two-step oxidation-reduction sequence was used to give the desired 6-*O*-methyl mannose acceptor **106** (85% yield). Change in the $^3J_{1,2}$ coupling constant (from 7.6 to 1.0 Hz) indicated inversion to the β-*manno*-configuration.



Scheme 2.36. *Synthesis of methyl 6-O-methyl-β-D-mannose acceptor (106).*

Reaction of the 6-deoxy **101** and 6-*O*-methyl **106** acceptors with benzylated trichloroacetimidate[175] donor **21** under TMSOTf activation gave disaccharides **107** (96% yield) and **110** (68% yield), respectively (Scheme 2.37). The disaccharides were then treated with NaOMe/MeOH followed by the standard oxidation-reduction sequence to provide intermediates **109** and **112**.

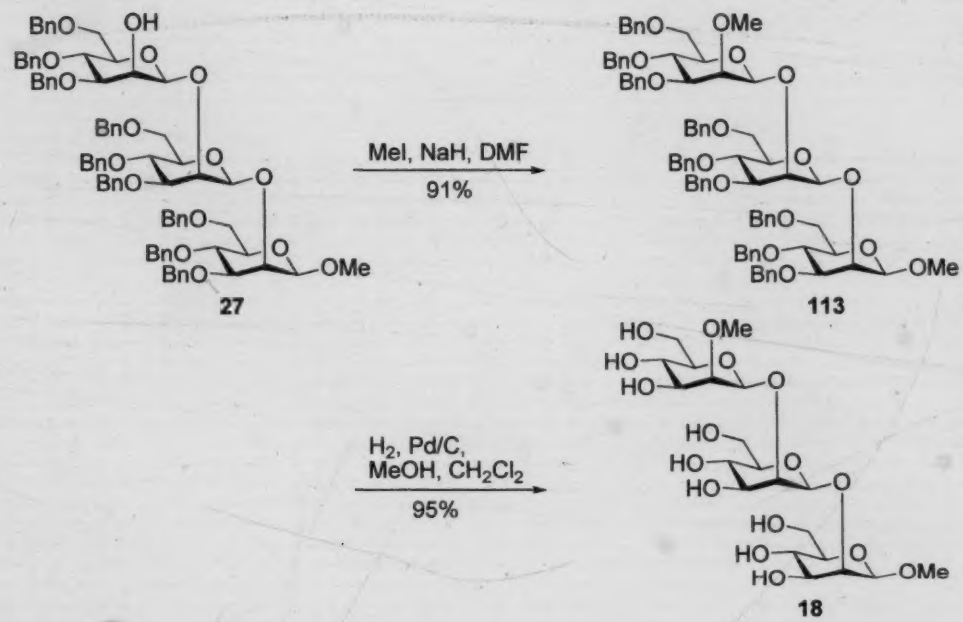


Scheme 2.37. *Synthesis of 6-deoxy (16) and 6-O-methyl (17) analogues.*

Subsequent hydrogenolysis of **109** and **112** gave the desired 6-deoxy **16** (86% yield) and 6-*O*-methyl **17** (69% yield) disaccharide analogues respectively. The $^{1}J_{C\text{-}1,H\text{-}1}$ and $^{3}J_{1',2'}$ coupling constants confirmed the β-*manno* configuration for both **16** and **17**.

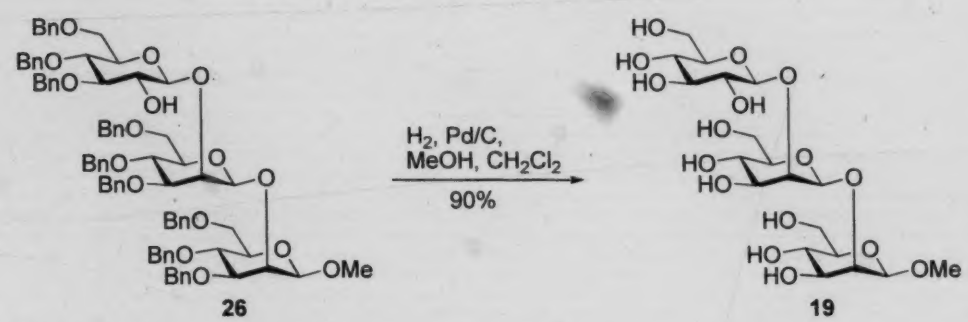
2.2.10 Synthesis of C-2″ modified trisaccharide analogues.

Intermediate **27**, used in the synthesis of native trisaccharide **3**, was methylated using methyl iodide and sodium hydride to give **113** in 91% yield (Scheme 2.38). Global debenzylation gave the desired 2″-*O*-methyl trisaccharide analogue **18** (95% yield). Analysis of the HMBC spectra showed correlation between H-2″ (3.88 ppm) and methyl carbon (62.6 ppm) indicating methylation of the C-2″ hydroxyl group.



Scheme 2.38. *Synthesis of 2″-O-methyl trisaccharide analogue (18).*

The 2''-*gluco* trisaccharide analogue **19** was prepared from intermediate **26** in 90% yield by catalytic hydrogenation (Scheme 2.39). Overlap of the resonance for H-1'' in the ^{1}H-NMR with the HOD signal, precluded characterization of the 1,2-stereochemistry of the terminal non-reducing sugar residue.



Scheme 2.39. *Synthesis of 2''-gluco trisaccharide analogue (19).*

2.3 Conclusions.

We have synthesized a panel of complementary deoxy and *O*-methyl analogues of the immunologically important (1→2)-β-D-mannopyranoside. Where possible hydroxyl group modifications were conducted at the monosaccharide stage to reduce the chemical steps performed on the valuable disaccharides. The challenging (1→2)-β-D-*manno* linkage was approached by a two-step oxidation-reduction sequence following highly β-stereoselective glucosylation employing glucosyl donors with neighbouring group participation. These final structures will serve as value tools to probe the key polar contacts involved in the binding of the known protective mAb C3.1 (IgG3).

Chapter Three

Effects of functional group modification on the binding of (1→2)-β-D-manno-pyranosides by mAb C3.1:

3.1 The ELISA protocol.

The enzyme-linked immunosorbent assay (ELISA) is a simple, versatile and sensitive technique for studying antigen and antibody interactions. It has become one of the most widely used immunological assays and can be employed for analysis of a single sample or high-throughput screening.[212] Though there are several ELISA formats all involve the specific interaction between antigen and antibody, with one of the reactants immobilized to a solid support.

In competitive ELISA, micro-titre plates are coated with a known antigen then a fixed amount of antibody is incubated with varying concentrations of soluble antigen. In the absence of soluble antigen, the antibody associates freely with solid phase antigen. However, in the presence of soluble antigen the antibody is partially blocked from binding to the solid phase. Following equilibration, antibody that is not bound to the solid phase is removed by washing and the remaining antibody is detected by the use of an enzyme-conjugated reagent together with a substrate capable of generating a coloured reaction. In the direct competitive ELISA the primary antibody is conjugated directly to an enzyme. For the indirect competitive ELISA a secondary enzyme-conjugated antibody is used for detection (i.e. goat anti-mouse IgG antibody). Advantages of the indirect method include; (1) a wide variety of labelled secondary antibodies are commercially available, (2) the same labelled secondary antibody can be used for detection of many different primary antibodies made in the same species, (3) using a

labelled secondary antibody avoids possibly affecting the immunoreactivity of the primary antibody, (4) sensitivity is increased because the primary antibody contains several epitopes recognized by the secondary antibody, allowing for signal amplification. Common enzymes used in ELISA for conversion of a colourless substrate to a coloured product include horseradish peroxidase, alkaline phosphatase and β-galactosidase.

The inhibition of antibody binding to the solid phase is proportional to both the quantity of antigen in solution and the antigen-antibody affinity. Inhibition curves for a specific antigen can be generated by determining the inhibition of a fixed amount of antibody with varying concentrations of soluble antigen in serial dilution. Subsequently, an inhibition curve can be used to quantify the amount of known antigen in a specific solution by relating percent antibody-inhibition to antigen concentration or assess antibody specificity by comparing the inhibitory power of soluble antigens. Commonly, the concentration required for affecting 50% inhibition of antibody binding to the solid phase (IC_{50} value), calculated from the inhibition data, is used to compare closely related antigens. Antigens having lower IC_{50} values possess higher affinity to the antibody. While it is expected that compounds having little affinity to the antibody will have high IC_{50} values, these values may be outside the measured concentration range. Though in some cases the tested concentration range may be adjusted, it may be inconvenient and a waste of precious synthetic inhibitor to measure the true IC_{50} value for poorly binding substrates and often values are quoted as being greater than the highest concentration tested.

The specific protocol used for studying inhibition of mAb C3.1 with synthetic analogues of (1→2)-β-D-mannopyranosides was developed by Bundle and is an indirect

competitive ELISA (Scheme 3.1).[139] Microtitre 96-well plates were pre-coated *via* non-covalent attachment of a fixed amount of BSA linked (1→2)-β-D-mannopyranoside. Monoclonal antibody mixed with synthetic soluble inhibitor in serial dilution was then added to the wells in triplicates. After incubation and washing steps a secondary antibody, goat anti-mouse IgG antibody conjugated to horseradish peroxidase, was added. The enzyme substrate, 3,3′,5,5′-tetramethylbenzidine, was introduced and oxidation by HRP produced a blue solution. Quenching the reaction with phosphoric acid resulted in a yellow solution that was then measured spectrophotometrically at 450 nm. Generally, as the concentration of the soluble inhibitor increases, a decrease in the color and absorbance reading is observed. The absorbance readings of the wells are corrected against a control (absorbance measured from wells containing only media) and the data is plotted as % inhibition *vs.* $\log_{10}$ of the ligand concentration. The IC_{50} is then determined from the concentration of ligand that reduces by 50% the absorbance of the control which contained no ligand.

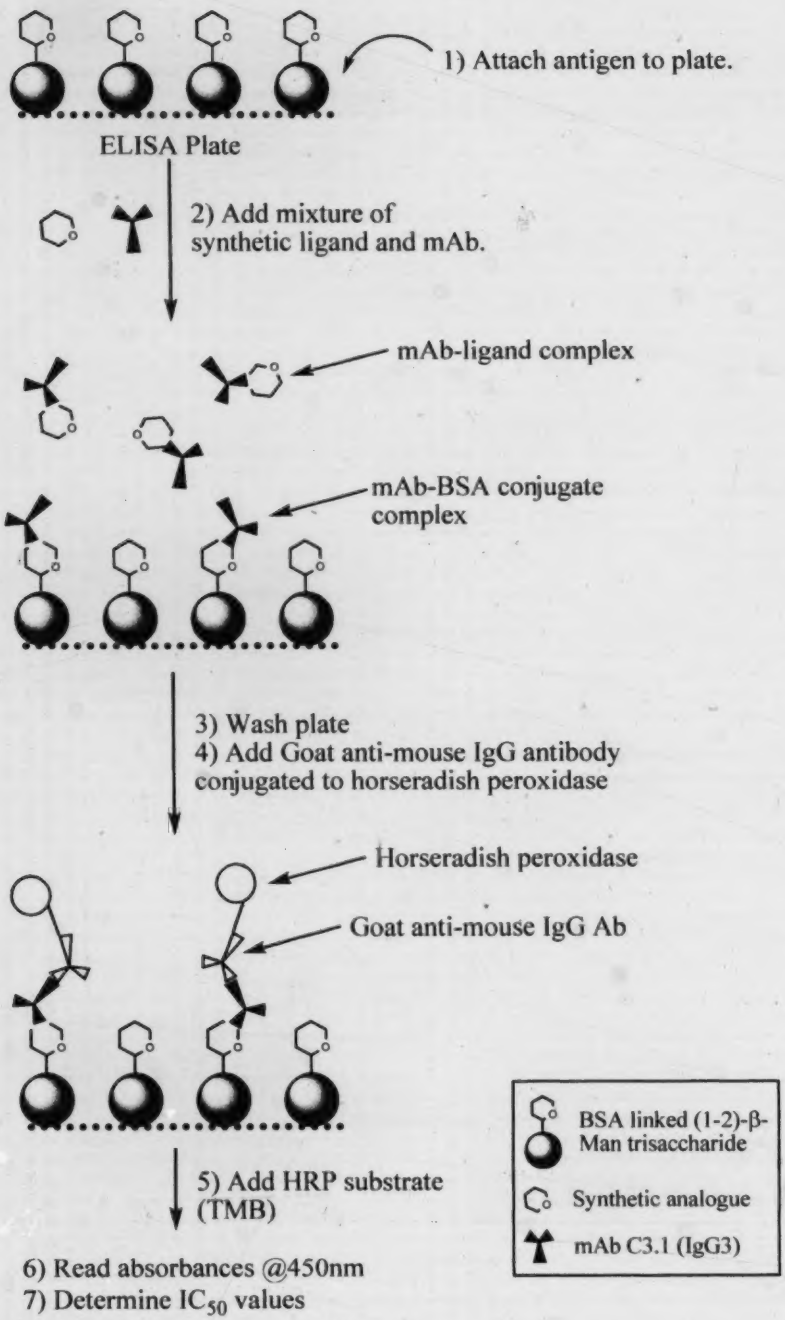


Figure 3.1. *Indirect competitive ELISA protocol used for evaluation of synthetic analogues against mAb C3.1 (IgG3).*

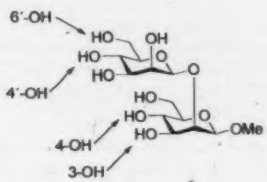
3.2 Mapping the protective epitope recognized by mAb C3.1.

The affinity of mAb C3.1 (IgG3) for the synthesized methyl glycosides (**1, 2** and **4-19**) was determined in an indirect competitive ELISA. Each synthetic analogue was tested in triplicate over a range of concentrations to provide inhibition curves and IC_{50} values (Table 3.1. and 3.2.). The inhibitor activity of synthetic analogues **4-19** were assessed by comparing their IC_{50} values to those obtained for the reference inhibitors, disaccharide **1** or trisaccharide **2**. Differences in the compared IC_{50} values provided a means to determine the extent of involvement of the hydroxyl groups in the binding of the native (1→2)-β-D-mannopyranoside with the mAb C3.1.

The reference inhibitor, native disaccharide **1**, showed almost 4 times worse activity (IC_{50} 31 μmol/L; Table 3.1.) against mAb C3.1 than was previously reported for the disaccharide propyl β-D-mannopyranosyl-(1→2)-β-D-mannopyranoside (IC_{50} 8 μmol/L; Table 1.1.).[139] However, trisaccharide **2** showed similar inhibitor activity (IC_{50} 17 μmol/L; Table 3.1.) to a previously tested propyl trisaccharide (IC_{50} 16 μmol/L; Table 1.1.). The reasons behind this switch in the seeming preference of the antibody for the trisaccharide over the disaccharide are not known, however, slight differences existed between the present and former study, including inhibitor aglycone and antigen used to coat the ELISA plates. In the present work the inhibitors contained a methyl aglycone and plates were coated with a (1→2)-β-D-mannan trisaccharide-BSA conjugate, while in the former study the inhibitors contained a propyl aglycone and the ELISA plates were coated with isolated PMC. Importantly however, the magnitudes of the IC_{50} values for the di- and trisaccharides against mAb C3.1 measured in the two studies were similarly in the low μmol/L range.

Table 3.1. *The effects of hydroxyl group modification on the inhibition by disaccharide* **1** *of the binding of mAb C3.1 (IgG3) to solid supported (1→2)-β-D-mannan trisaccharide-BSA conjugate.*

Compound	Derivative	IC_{50} μmol/L	Relative Potency %	Δ(ΔG) kcal/mol
1	*disaccharide*	31	100	0
4	2′-*O*-methyl	33	94	0.04
5	2′-*gluco*	47	66	0.3
6	3′-deoxy	119	26	0.8
7	3′-*O*-methyl	62	50	0.4
8	4′-deoxy	Inactive†	<1	>2.7
9	4′-*O*-methyl	670	5	1.8
10	6′-deoxy	426	7	1.6
11	6′-*O*-methyl	588	5	1.7
12	3-deoxy	Inactive†	<1	>2.7
13	3-*O*-methyl	Inactive‡	<1	>2.6
14	4-deoxy	Inactive†	<1	>2.7
15	4-*O*-methyl	Inactive‡	<1	>2.6
16	6-deoxy	14	221	-0.5
17	6-*O*-methyl	81	38	0.6
2	*trisaccharide*	17	182	-0.4
18	2″-*O*-methyl	56	55	0.4
19	2″-*gluco*	52	60	0.3



† No inhibition at 2938 μmol/L
‡ No inhibition at 2700 μmol/L

The ELISA data clearly demonstrated that modification of certain hydroxyl groups on disaccharide **1** significantly decreased inhibitor activity (Table 3.1.). The relative potencies of compounds **8-15** were drastically reduced compared to **1**. Several modifications (**8** and **12-15**) gave compounds with estimated IC_{50} values greater than their measured concentration ranges; these increases were considered very significant and the compounds were defined as being inactive. Modification of the 4′-OH (**9**) or 6′-OH (**10** and **11**) gave measurable IC_{50} values; however, the reduction in potency remained significant. These results indicate the important and direct involvement of the 3-OH, 4-OH, 4′-OH and 6′-OH in the binding of **1** to the antibody.

Interestingly, compound **7** (3′-*O*-methyl) was only a two times worse inhibitor than **1**. Though, this reduction in activity is certainly not large enough to indicate direct involvement of the 3′-OH in binding it suggests the hydroxyl group is on the periphery of the binding site. A marked difference in inhibition is observed between compounds modified on the 4′-OH or 3′-OH thus defining a boundary for the epitope contained in **1**. A similar epitope boundary between the 4-OH and the neighbouring 6-OH was apparent from the inhibition data. While compounds **14** and **15** were completely inactive, the inhibition data for **16** and **17** indicates there is some allowance for modification of the 6-OH. The 6-*O*-methyl compound **17** was about a two and a half times worse inhibitor than **1**, suggesting this hydroxyl lies along the periphery of the binding site. Remarkably, the 6-deoxy compound **16** was found to be a two-fold better inhibitor than **1**. The origin of this increased inhibitor activity for **16** is unknown. A similar situation has been observed for inhibition with other 6-deoxy compounds.[102,213-215] In these instances the superior inhibitor activity of the deoxy compounds was rationalized by the requirement for

formation within the binding site of an intramolecular hydrogen bond with the 6-OH of the parent compounds.

Methylation of the 2′-OH **4** did not affect inhibitor activity. Addition of this terminal methyl group on the disaccharide was not expected to provide much steric repulsion within the binding site or affect the binding greatly as the addition of a much larger third sugar residue provided a marginal increase in binding (see **2**). Compound **5**, the 2′-*gluco* epimer of **1**, did not show significant change in inhibitor activity.

Currently, the conformation adopted by a (1→2)-β-D-mannopyranan bound to mAb C3.1 is unknown. However, the studied solution conformation of synthetic oligomers of (1→2)-β-D-mannopyranans shows a helical structure with a repeating unit of approximately 3 residues long.[139] The tri-, tetra- and pentasaccharides have been found to explore very similar torsional angles across all their linkages. Considering the structural conformity and rigidity of the oligomers it seems a reasonable assumption that the conformation of the native (1→2)-β-D-mannopyranan on the fungal surface and that recognized by mAb C3.1 would adopt a similar helical structure. In the absence of other conformational data, the solution conformation served as an essential reference point for interpretation of inhibition data.

The global minimum energy model of a disaccharide portion of a (1→2)-β-D-mannopyranan correlates well with the inhibition data exhibited by the synthetic analogues. The inhibition data indicated that modification of the 3-OH, 4-OH, 4′-OH or 6′-OH resulted in complete abolition of inhibitor activity. From the model it appears that these hydroxyl groups, essential for ligand binding, are located along one edge of the helical epitope. Additionally, the 6-OH, 2′-OH and 3′-OH which were found to be non-

essential for complex formation are shown oriented opposite to this binding region. Together this data supports a groove type binding site with one edge of the antigen making contact with the antibody, while the remainder of the ligand is exposed to solvent. A cavity-like binding site might require stronger binding from the more terminal hydroxyl groups such as the 2'-OH. Also, the contact surface of a ligand binding within a cavity would not be expected to be limited to an edge of the antigen.

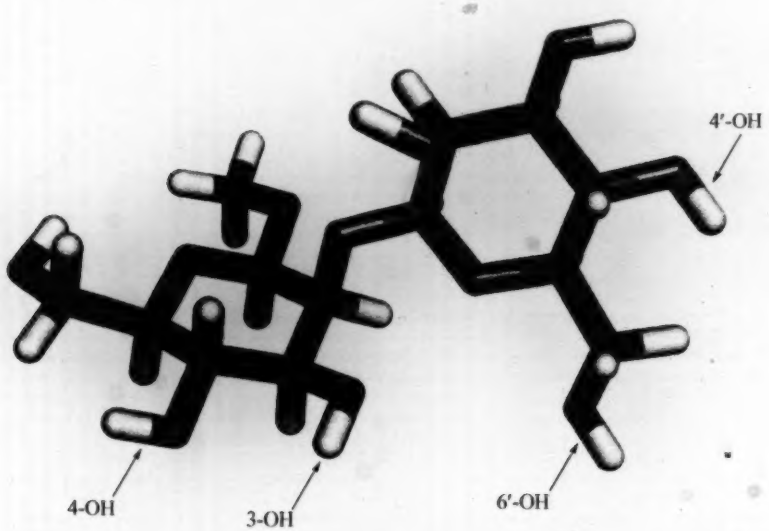


Figure 3.2. *Global minimum energy model of a disaccharide portion of a (1→2)-β-D-mannopyranosyl undecasaccharide. The hydroxyl groups at C-3, C-4, C-4' and C-6' essential for binding are labelled and appear along one edge of the helical epitope.*

Predicting the binding epitope from mapping studies using an extensive panel of modified oligosaccharides to identify important protein-carbohydrate interactions has been proven successful with the general agreement between the epitope map and co-crystal structure of *Shigella flexneri* lipopolysaccharides and antibody SYA/J6.[216,217] The model should provide an understanding of the size and topography of the bound ligand as

well as identify any key polar contacts essential for binding. The present study used a panel of synthetic complimentary mono-deoxy and mono-*O*-methyl disaccharide analogues of (1→2)-β-D-mannopyranan to chemically map the epitope recognized by the protective mAb C3.1. Though the bound conformation of the ligand is unknown, an available solution conformation of the (1→2)-β-D-mannopyranan has proven useful in identifying a continuous contact surface for the disaccharide. Particularly, four hydroxyl groups have been shown to form hydrogen-bonds essential for complex formation.

In considering the size of the antigen binding site it should be recalled that the mAb was generated against the native PMC. Further, Cutler's work showed that the antibody recognized the acid labile component, i.e. the (1→2)-β-D-mannan linked via the phosphate to the acid stable mannan. While that experiment may not be definitive, in the absence of contradictory data we conclude that the acid labile mannan is the target antigen that elicited the antibody. Bundle and Nitz found that the tetrasaccharide (IC_{50} 84 μmol/L) was about a five times worse inhibitor than the trisaccharide (IC_{50} 16 μmol/L).[139] The inhibitor activity dropped off considerably with the pentasaccharide (IC_{50} 421 μmol/L). In the present work, trisaccharide **2** was found to be a near two-fold better inhibitor than disaccharide **1**, suggesting that the upper size limit of the antibody-binding site accommodates a trisaccharide (Table 3.1). In terms of energy, however, addition of the third sugar residue resulted in only a marginal effect on binding suggesting that the bulk of the binding energy is contributed by a disaccharide portion of the trisaccharide. The ELISA inhibition data from the modified analogues identified a continuous contact surface located along one edge of the disaccharide. Considering this, there are then two potential frame-shifted binding modes predicted for extrapolation of the contact surface

onto the trisaccharide (Figure 3.3). In the first instance the binding region is mapped onto the two terminal reducing end residues (**A**) and in the second the two terminal non-reducing residues (**B**). As discussed above if the antibody is assumed to be raised against the acid labile phosphomannan then any binding model for recognition of the antigen should be consistent with presentation of the (1→2)-β-D-mannan attached to the intact phosphomannan molecule via an α-linkage.

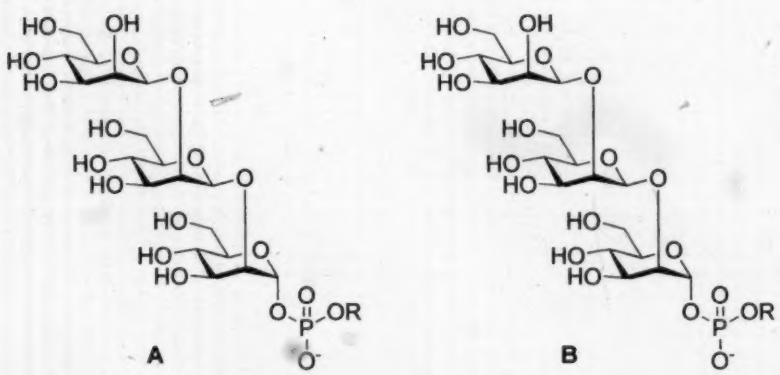


Figure 3.3. *Two available frame-shifted binding modes for a disaccharide occupying the binding site of mAb C3.1. The disaccharides are highlighted in blue indicating the binding mode for the continuous contact surface.*

Extrapolation of the continuous contact surface on to a global minimum energy structure of trisaccharide **2** provides a model for distinguishing one of two potential binding modes (Figure 3.4). Binding modes (a) and (b) represent two different orientations of the same global minimum energy structure. In binding mode (a) the recognition elements from the continuous contact surface are labelled on the terminal reducing disaccharide residues (Figure 3.4a). In the model the terminal reducing residue is shown forming a β-linkage to the methyl aglycone, however, in the native structure if this trisaccharide is attached to the acid labile portion of the PMC it would connect via an

α-linkage to the remainder of the complex. Since the key hydroxyl groups crucial for recognition are on the same face this would present the α-phosphate linkage and the remainder of the acid stable mannan unfavourably to the antibody. This argument would appear to exclude recognition of the terminal reducing disaccharide unless this (1→2)-β-D-mannan structure is linked directly to the α-mannan chains on the acid stable portion of the PMC *via* a β-glycosidic linkage (as shown in Figure 1.6). In binding mode (b) the recognition elements are labelled on the terminal non-reducing disaccharide residues (Figure 3.4b). This binding mode would be consistent with the contact surface as part of the acid labile (1→2)-β-D-mannan since the terminal reducing residue is not involved with the binding site and the large phosphomannan would not interfere with the recognition of the crucial disaccharide epitope. Interestingly, in both models the terminal residue that is not part of the extrapolated contact surface is oriented opposite to the binding surface suggesting that in either case this residue would contribute little to antibody binding.

The 2″-*O*-methyl and the 2″-*gluco* compounds **18** and **19** were each about three times worse inhibitors than the parent trisaccharide **2** (Table 3.1). Though, it is not clear whether the 2″-hydroxyl forms hydrogen bonds important for binding, it seems that introduction of the methyl group provides enough steric repulsion to disrupt binding. The 2″-hydroxyl group likely lies along the periphery of the binding site.

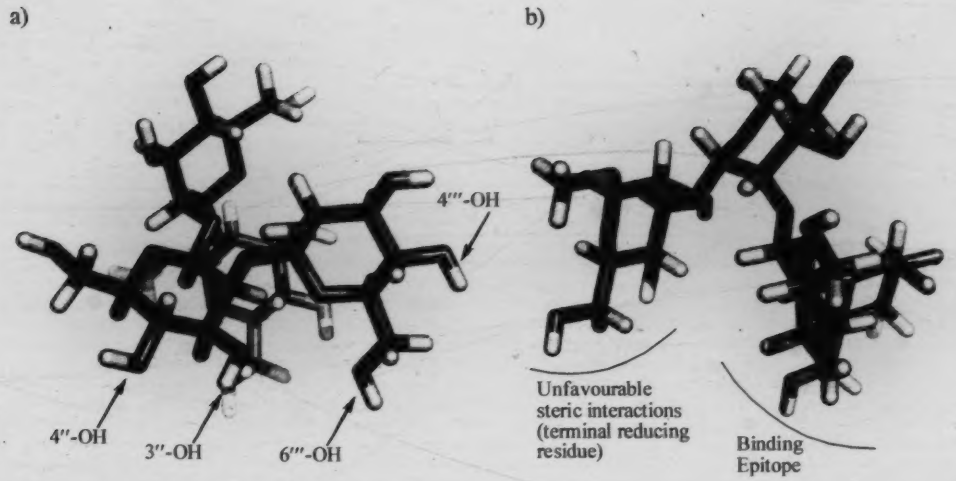


Figure 3.5. *Global minimum energy model of a tetrasaccharide portion of a (1→2)-β-D-mannopyranosyl undecasaccharide. a) The continuous contact surface extrapolated over the terminal non-reducing disaccharide residues of the tetrasaccharide. b) Side view of tetrasaccharide model showing potential unfavourable steric interactions with the antibody.*

In the following Chapter, studies toward the use of saturation transfer difference-NMR to study the trisaccharide binding to mAb C3.1 are described. This method can potentially identify which portions of the ligand are in closer contact with the antibody and therefore possibly define the preferred binding mode of the disaccharide. In Chapter 6, conclusions and discussions, further experiments are proposed to clarify the unresolved questions relating to the mode of binding.

Chapter Four

Qualitative analysis of trisaccharide binding to mAb C3.1 using saturation transfer difference NMR:

4.1 General introduction to saturation transfer difference NMR.

Saturation transfer difference (STD) NMR was developed in 1999 by Mayer and Meyer.[218] Since then the technique has been used to study carbohydrates, glycopeptides, peptides and drug-like compounds.[219-229] The STD-NMR experiment involves selective irradiation of protein resonances with radio frequency energy and relies upon efficient intermolecular transfer of magnetization to the bound ligand (Figure 4.1). The magnetization transferred is dependent on the proximity of the ligand surface to the protein surface. Ligand protons that are more closely bound receive the greatest amount of magnetization transfer while protons exposed to bulk solvent receive the least amount. The on-resonance protein irradiation spectrum is internally subtracted from a control spectrum by the NMR instrument and the outcome is a one-dimensional (1-D) spectrum displaying ligand resonances, generated by difference spectroscopy. The intensities of the individual proton resonances arise from the amount of magnetization transferred to the ligand. Protons receiving irradiation during the on-resonance pulse observe a decrease in signal intensity dependent on the amount of saturation; because of the difference spectroscopy protons which are more closely bound and that receive the greatest amount of saturation transfer will appear with greater intensity in the NMR spectrum. Ligand resonances that receive the least amount of magnetization transfer appear the smallest, while, non-binding molecules are completely subtracted from the

spectrum. The STD-NMR experiment thus provides a means to map the epitope recognized by the protein.

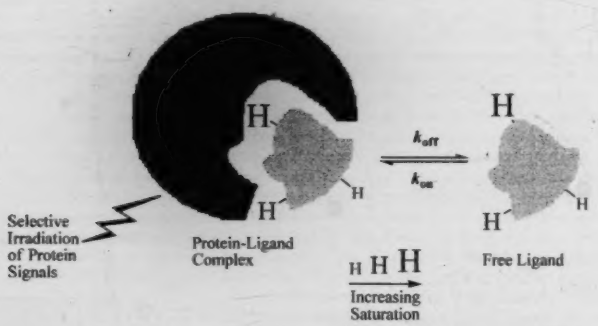


Figure 4.1. *Illustration of epitope mapping using the technique of saturation transfer difference NMR. Selective irradiation of the protein results in the transfer of magnetization to a ligand which is in fast exchange between the bound and free states.*[230]

Selective irradiation of the protein is possible because protein resonances have significant intensity outside the normal spectral window of low-molecular weight compounds. Protein resonances can extend from the negative parts-per-million (ppm) region to downfield above 10 ppm. Once the protein is irradiated there is very efficient intramolecular transfer of magnetization throughout the entire protein due to spin-diffusion which leads to fast saturation of the protein.[230,231]

Systems suitable for studying with STD-NMR have ligand dissociation constants K_D ranging from ca. 10^{-3} to 10^{-8} M. The appearance of ligand resonances depends greatly on fast exchange between the bound and free states. Ultimately, STD-NMR depends on the on- and off-rates of the ligand where k_{on} and k_{off} represent the rates of association and dissociation of a ligand and its receptor (Figure 4.1). The dissociation rates can be estimated by a assuming a diffusion controlled association where k_{on} is about 10^7 $s^{-1} \cdot M^{-1}$.

If dissociation of the ligand from the receptor is too slow on the NMR time-scale, the ligand will not be released sufficiently for detection. If the dissociation is too fast, the amount of time that the ligand is in contact with the protein maybe insufficient for magnetization transfer to occur. Thus, even in a mixture of substances the ligand can be detected if the binding kinetics are appropriate. Binding components are clearly visible in the 1-D spectrum as non-binding components are effectively eliminated during the difference spectroscopy. Though, the kinetics and equilibrium constants for the (1→2)-β-D-mannan trisaccharide and mAb C3.1 (IgG3) system are not known, the IC_{50} value for trisaccharide **2** (17 μmol/L) from ELISA inhibition data estimates a dissociation constant K_D of 10^{-5} M.

Another advantage of STD-NMR is that due to its high sensitivity the experiment can be performed with as little as 1 nmol of protein with molecular weight >10 KDa. Typically, a 100-fold excess of ligand is used to maximize signal to noise. The spectra can be acquired within minutes or hours depending on protein concentration and the affinity of the ligand.

4.2 Features of the STD-NMR experiment.

The STD-NMR experiment involves the successive on-resonance STD excitation of the protein followed by collection of the off-resonance difference spectrum.[230] The selective on-resonance pulse is placed within the region of the protein resonances but at a sufficient distance from ligand resonances so that the ligand is not directly affected. The difference spectrum is acquired by selective off-resonance irradiation. Typically, a large value ~30 ppm is selected for off-resonance irradiation, although any value that does not affect either the protein or ligand resonances is suitable. Subtraction of the on-resonance

and off-resonance spectrum is performed internally by the NMR software *via* phase cycling after every scan to minimize artifacts due to temperature and magnet instability. The total saturation time can range from 250 ms to 2.5 s without affecting the STD spectra. Signal-to-noise is increased with longer magnetization times.[232] Due to the non-uniformity of T_1 proton relaxation of the ligand longer magnetization times should be avoided. For example, selective pre-saturation of the protein can be achieved by a train of 30 gradient pulses each being 50 ms in duration to give a total magnetization time of 1.5 seconds. Following the selective pulse cascade, a full 90° pulse is applied affecting all of the resonances in the spectrum. This is termed the "read pulse' and is necessary for proper subtraction of data. The remainder of the pulse sequence includes a spin-lock pulse ($T_1\rho$ filter) that removes signals arising from the broad protein resonances, water suppression, followed by recording of the free induction decay (FID).

A reference NMR spectrum is acquired using the same sample that was used to obtain the STD-NMR spectrum and is essentially a 1D spectrum containing all ligand resonances. The same pulse sequence used to collect the STD spectrum is used for the reference spectrum with a few changes. The so-called on-resonance pulse is now set to equal the off-resonance pulse (e.g. 30 ppm). The difference function is now turned off and no data will be subtracted following successive scans. Importantly, the length of each scan remains the same for both the STD and reference experiments, however, because no subtraction of on-resonance/off-resonance data occurs during acquisition of the reference spectrum the total number of scans is divided in half. The remainder of the pulse sequence remains the same including the spin-lock and water suppression.

The intensities of both the STD and reference spectra are measured using the NMR software. The absolute intensity of each resonance is measured *via* a linear vertical scale and only those having no overlapping signal can be used. The intensity of each individual peak is converted to the same vertical scale and then converted to STD amplification factors (η) *via* the following equation:

$$\eta = I_o - I_{sat}/I_o \times \text{ligand excess}$$

where $I_o - I_{sat}$ is the intensity of the saturation transfer effects from the difference spectra and I_o is the intensity of the same peak in the reference spectra. The amplification factors are then expressed as percentages with the highest value normalized to 100%. The same normalization parameter is used to scale the remaining amplification factors. Together the values are used to describe the epitope of the ligand recognized by the protein.

To ensure that the signal in the STD-NMR is authentic a control experiment should be run under identical conditions on a sample containing only ligand. With the on- and off-resonance pulse set accordingly ligand resonances should be absent in the difference spectrum.

4.3 Optimizing STD-NMR pulse sequence parameters.

The first use of the STD-NMR technique in the Department of Chemistry at the University of Alberta was employed for the study of *S. flexneri* trisaccharide congeners. The pulse sequence used for that system provided an appropriate starting point for determining the optimal parameters for studying the binding of (1→2)-β-D-mannopyranan trisaccharide and mAb C3.1 (IgG3). The position and selectivity of the protein saturation cascade had to be adjusted as it was found that the initial parameter values directly affected the ligand resonances. Also, it was necessary to further adjust the

pulse width for the water suppression sequence to be as narrow as possible without affecting the ligand resonances. Considerable effort was made to further optimize the pulse sequence. Figure 4.2 shows the STD-NMR pulse sequence.

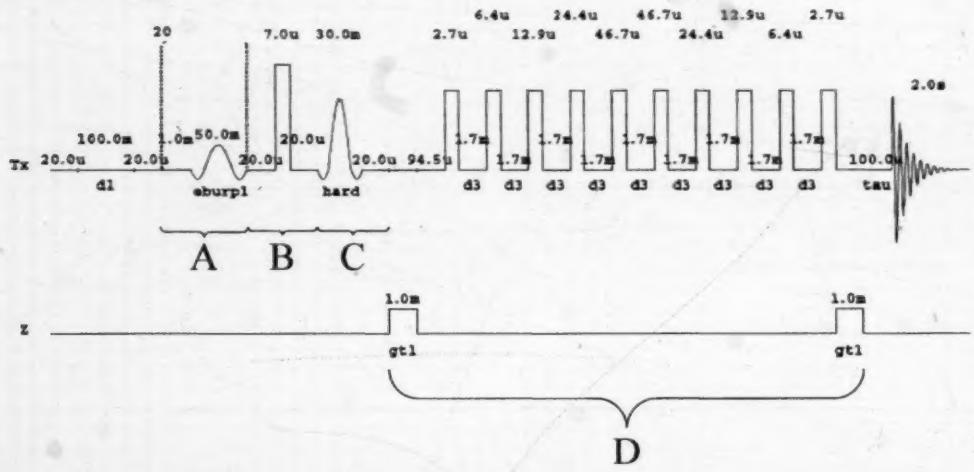


Figure 4.2. *The STD-NMR pulse sequence. (A) The selective irradiation cascade. The on-resonance pulse was set to -1.3 ppm and the off-resonance pulse was set to 35.0 ppm. (B) The 90° read pulse. (C) The $T_{1\rho}$ filter. (D) Watergate W5 water suppression sequence. Times are measured in units of s (seconds), m (ms, milliseconds) or u (μs, microseconds).*

4.3.1 Placement of the selective on- and off-resonance pulse cascades.

To determine the optimal position for the placement of the selective on- and off-resonance pulses a solution of only the ligand in deuterium oxide was subjected to a 1-D TROESY experiment. The advantage of using the TROESY was that the selective pulse frequency could be manipulated as the single variable. Arrays of spectra were acquired using the EBURP1 pulse profile at various frequencies to determine the selective on-resonance (Figures 4.4 and 4.5) and off-resonance (Figures 4.6) pulses. Any visible ligand peaks are the result of direct irradiation from the selective pulse. The EBURP1

pulse profile is known to be an extremely selective pulse with a narrow width. Identical arrays of spectra were acquired using a Gaussian shaped pulse profile, however, this less selective pulse resulted in irradiation of ligand resonances at all of the frequencies screened (*data not shown*).

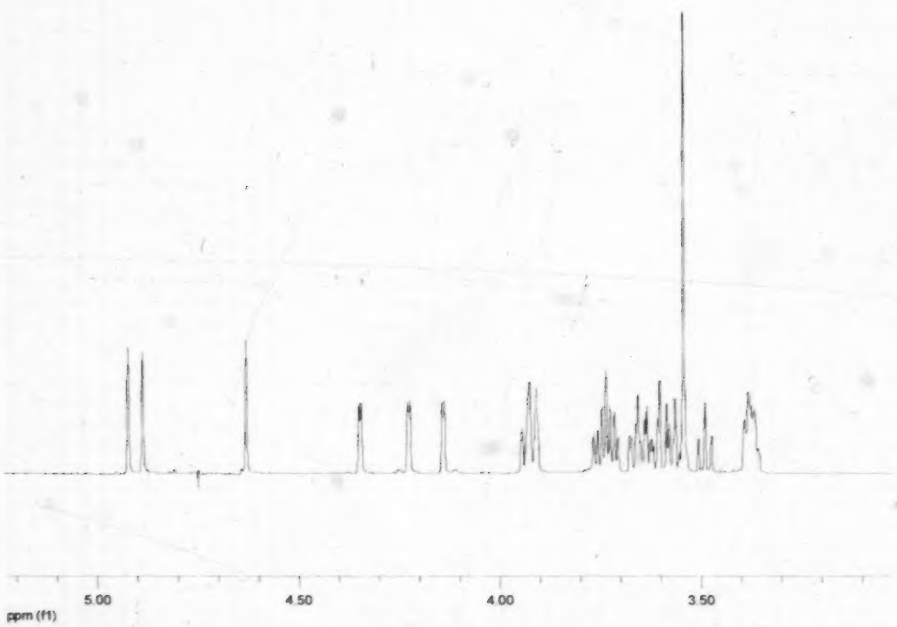


Figure 4.3. *1-D NMR spectra of trisaccharide* **2** *measured at 600 MHz.*

The 1-D NMR spectrum at 600 MHz of trisaccharide **2** shows ligand resonances ranging from 5.0 ppm to 3.3 ppm (Figure 4.3). Conveniently, the anomeric and C-2 protons from each residue are resolved. For selection of the optimal on- and off-resonance pulses frequencies were selected to avoid direct irradiation of the ligand protons.

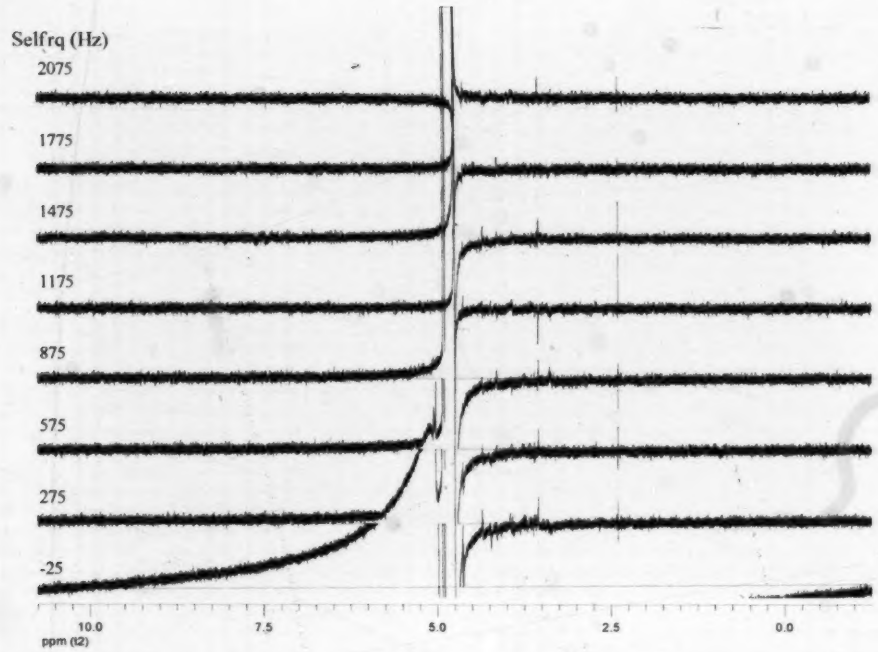


Figure 4.4. *Array of the EBURP1 on-resonance pulse in the aromatic region (64 scans each). Note the unacceptable ligand irradiation of the resonance at ~3.5 ppm that occurs from-25 Hz to 1475 Hz and 2075 Hz. Experiment measured at 600 MHz magnetic field strength.*

The arrays shown in Figures 4.4 to 4.6 are labelled in absolute spectrometer frequency (in Hz). The arrays were acquired by increasing the pulse frequency in 300 Hz (0.5 ppm) increments. Each spectrum was acquired at 600 MHz. In the aromatic region, 2075 Hz corresponds to 8.5 ppm and -25 Hz to 5.0 ppm. The spectra were recorder using a sample of trisaccharide **2** in deuterium oxide, with no antibody present. Ligand resonances are observed in the spectra when the selective pulse inadvertently irradiates the ligand. The additional intense sharp peak in the 1D spectrum (~2.5 ppm) is an artefact resulting from incomplete subtraction of data acquired during the on- and off-resonance pulses. The large water signal (~4.8 ppm) is also apparent.

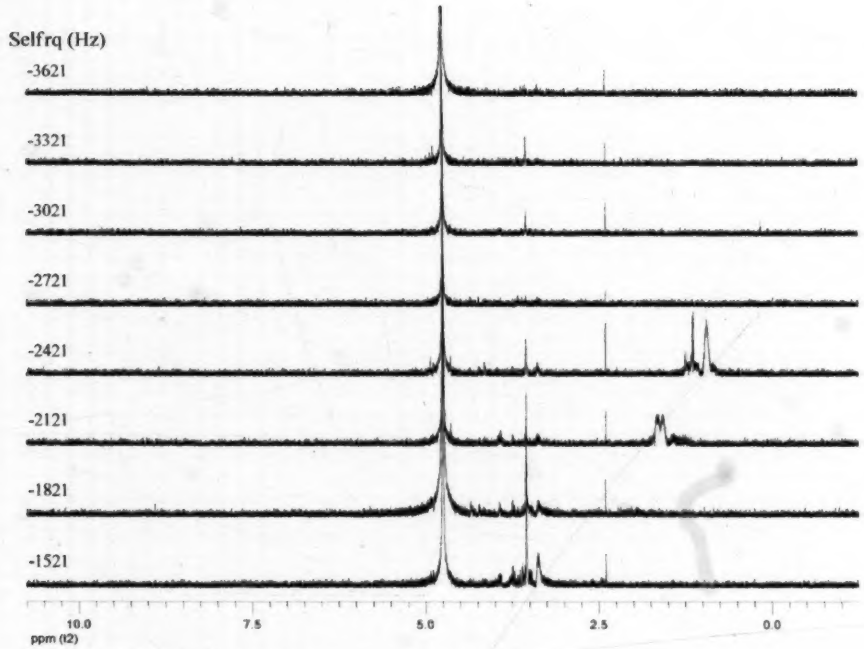


Figure 4.5. *Array of the EBURP1 on-resonance pulse in the aliphatic region (64 scans each). Note the unacceptable ligand irradiation of the resonance between ~3.25 and 4.0 ppm that occurs throughout the arrays. This irradiation was absent at -2721 and -3621 Hz. The resonance at 2.5 ppm is from irradiation of an impurity in the sample. Experiment measured at 600 MHz magnetic field strength.*

Application of a selective pulse in the aliphatic region clearly demonstrates irradiation of the ligand resonances (Figure 4.5). In the aliphatic region, -1521 Hz corresponds to 2.5 ppm and -3621 Hz to -1.0 ppm.

Though the EBURP1 pulse profile is known to be more selective than the Gaussian variant it was necessary to conduct arrayed experiments to determine the optimal frequencies for off-resonance selective pulses (Figure 4.6). Interestingly, even when the pulse frequency is set to 20000 Hz (38 ppm) ligand resonances are observed as a result of side-band irradiation.

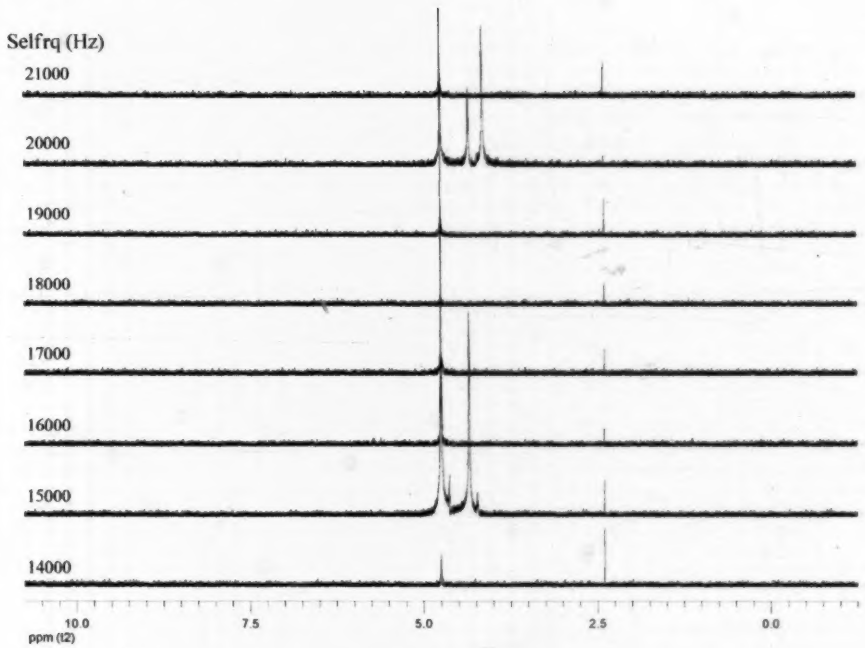


Figure 4.6. *Array of the EBURP1 off-resonance pulse in the far-aromatic region (64 scans each). Note the unacceptable ligand irradiation of the resonance at ~4.25 ppm that occurs in some of the arrays. This irradiation was absent with irradiation at 14000, 18000 and 19000 Hz.. The resonance at 2.5 ppm is from irradiation of an impurity in the sample. Experiment measured at 600 MHz magnetic field strength.*

For the on-resonance irradiation of the protein the selective pulse was set to -3621 Hz (-1.0 ppm) and the off-resonance pulse was set to 18000 Hz (35 ppm). Though the TROESY experiment conveniently allowed the optimization of the selective pulse frequency for the on- and off-resonance pulses it was important to test these frequencies in a true STD-NMR pulse sequence on a sample of the ligand alone to ensure no ligand resonances are irradiated (Figure 4.7). Unfortunately, after 7K scans using the pulse sequence shown in Figure 4.2 ligand resonances were observed in the spectrum collected from a sample containing the ligand only. The presence of ligand resonances could be an indication of ligand irradiation and/or result from the difference spectroscopy due to

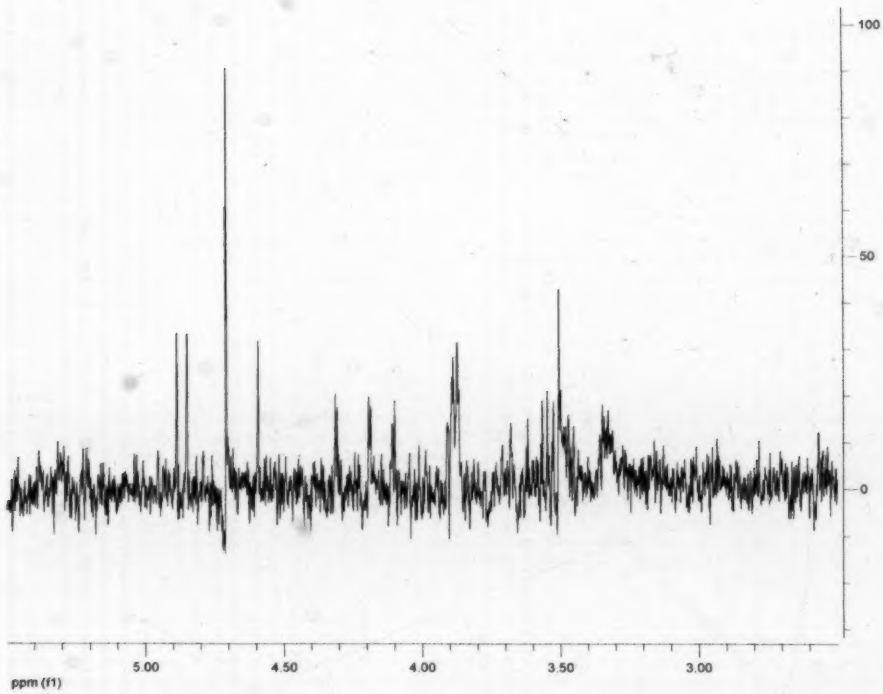


Figure 4.7. *Control STD-NMR spectrum of trisaccharide* **2** *measured at 600 MHz. The selective on-resonance pulse was set to -3621 Hz (-1.0 ppm) and the off-resonance pulse to 18000 Hz (35 ppm). Note the unacceptable appearance of trisaccharide resonances after acquisition of 14K scans.*

imperfections in the instrument and/or noise from the surrounding environment. It has been suggested that a control spectrum could be subtracted from the STD-spectrum to provide meaningful data;[233] however, this should only be performed if the ligand resonances in the control result solely from irradiation of the ligand and are not a symptom of problems with the difference spectroscopy. The resonances in the control spectrum were assumed to result from inadvertent ligand irradiation. The STD spectrum was corrected by subtraction of proton ligand intensities in the control from the STD spectrum. Identical peaks from each proton resonance in the STD, control and reference spectra were used to calculate STD amplification factors.

4.4 STD-NMR analysis of trisaccharide binding to mAb C3.1.

STD-NMR analysis was conducted on trisaccharide **2** with mAb C3.1. ELISA inhibition data has revealed that mAb C3.1 binds a trisaccharide with the majority of binding energy provided by a disaccharide portion. A continuous contact surface along one edge of the disaccharide was revealed and extrapolation onto the trisaccharide provided two potential frame-shifted binding modes. STD-NMR was expected to assist in further defining the epitope and mapping the continuous contact surface onto the trisaccharide. Antigen contact surfaces can be readily identified from signal amplification in the STD-spectrum. The surface of the trisaccharide that is most closely bound to the antibody could be differentiated from solvent exposed regions and lend support to one of the two possible frame-shifted epitopes.

Ligand proton resonances were observed in a control STD spectrum on a sample containing only ligand. As a result the normal quantitative approach to STD-NMR analysis could not be applied directly on the STD spectrum. The ligand resonance intensities in the control were lower than those in the STD-spectrum indicating authentic STD signals in the latter. A corrected STD spectrum was obtained by subtracting measured ligand intensities in the control from the STD spectrum to allow determination of a binding epitope (Figure 4.8).[233]

Conveniently, the three anomeric (4.5-5.0 ppm) and three C-2 proton (4.1-4.3 ppm) resonances of the trisaccharide ligand **2** are resolved in the 1D spectrum (Figure 4.8) and allowed construction of an epitope map (Figure 4.9). The H-2 proton of the terminal non-reducing residue receives the greatest amount of magnetization transfer and was scaled to 100%. A decrease in magnetization transfer is observed in H-2 protons

extending along the trisaccharide chain to the terminal non-reducing end residue, possibly suggesting that the terminal reducing end disaccharide portion makes the greatest contact with the protein. Saturation transfer is observed in the H-3 and H-4 proton resonances of each sugar residue but because of poor signal-to-noise in the STD and especially control spectra an amplification factor could only be calculated for H-4 of the terminal reducing-end residue. Also, H-6 (3.85-3.95 ppm) and H-5 (3.3-3.4 ppm) protons observed magnetization transfer, however because of extensive overlap in these regions it is not clear from which residue (possibly all three) these resonances originate. The aglycone methyl group also receives magnetization transfer. The broad signals at around 4.5 and 3.0 ppm are protein resonances that were not completely filtered.

Though the epitope map seems to suggest that the antibody binds to the terminal reducing end disaccharide of the trisaccharide ligand the data is not unambiguous because many of the ligand resonances could not be analyzed. It is possible that with this trisaccharide ligand these two-binding modes could be in equilibrium and the STD-NMR spectrum represents contribution from both. Comparison of the two global minimum orientations of the trisaccharide in Figure 3.4 indicates that both binding modes of the trisaccharide may be accessible to the antibody binding site because in each case the third terminal residue is oriented away from the contact surface. Therefore, without a more defined map including amplification factors from the remaining proton resonances the STD-NMR results do not conclusively discriminate between the two potential binding modes.

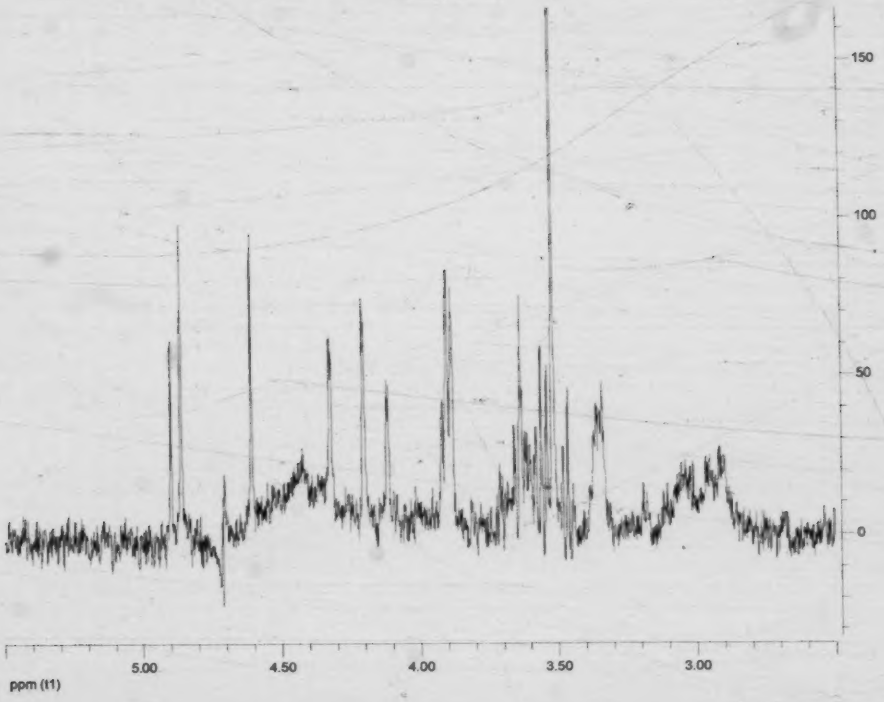
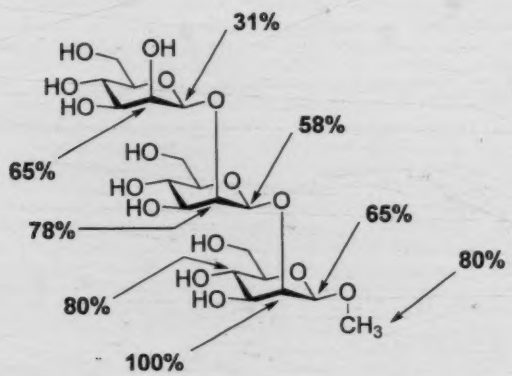


Figure 4.8. *STD-NMR spectrum of trisaccharide 2 with mAb C3.1 measured at 600 MHz. The selective on-resonance pulse was set to -3621 Hz (-1.0 ppm) and the off-resonance pulse to 18000 Hz (35 ppm) with 14K scans acquired.*



2

Figure 4.9. *The epitope map of trisaccharide 2.*

In summary, STD-NMR (600 MHz) was used to analyze the binding of trisaccharide **2** with mAb C3.1. Saturation transfer was observed in a corrected STD spectrum but due to extensive overlap of ligand resonances only a partial epitope map could be obtained. It appears that the terminal reducing end disaccharide is making the greatest contact with the protein but without a more complete epitope map, further comment on the binding mode of the trisaccharide should be avoided. Additional, STD experiments are proposed in Chapter 5 including repeating the analysis on trisaccharide **2** using a cryoprobe for better sensitivity and at higher field strength (i.e. 800 MHz) for greater signal dispersion.

Chapter Five

Conclusions and Future Directions

The research presented in this thesis describes efforts made to chemically map the (1→2)-β-D-mannopyranoside epitope recognized by the anti-*Candida albicans* protective monoclonal antibody C3.1 (IgG3). The aim was to better define the size and topology of the antibody binding site by identifying hydrogen bonds essential for ligand binding, to provide further insights into the structure of a minimal sized hapten that could be employed in a synthetic conjugate vaccine. To explore the key polar contacts an extensive panel of mono-deoxy, mono-*O*-methyl and epimeric di- and trisaccharide analogues of (1→2)-β-D-mannopyranoside were prepared and employed as inhibitors in a competitive ELISA experiment.

The synthesis of the analogues was achieved following a divergent route from advanced monosaccharide intermediates. The challenge of preparing the (1→2)-β-D-*manno* glycosidic linkage was met by employing a two-step oxidation-reduction sequence following highly selective β-*gluco* glycosylation employing neighbouring group participation. Competitive ELISA was used to evaluate the biological activity of all modified analogues. To identify key polar contacts the inhibitor activities of these analogues were compared against reference di- and trisaccharide ligands. The ELISA data indicated that hydroxyl groups at positions C-3, C-4, C-4′ and C-6′ are involved in forming hydrogen bonds essential to binding as any modification at these positions significantly decreased inhibition. A computer generated model of a disaccharide portion of the (1→2)-β-D-mannopyranoside shows that the essential hydroxyl groups are located

along one edge of the helical epitope. Furthermore, hydroxyl groups at positions C-6, C-2′ and C-3′ which were found to be non-essential to binding are found oriented opposite to this binding region. Together this data supports a groove type binding site with one edge of the antigen making contact with the antibody, while the remainder of the ligand is exposed to solvent. The antibody was predicted to bind a trisaccharide with the majority of binding energy provided by a disaccharide portion and thus extrapolation of the continuous contact surface onto the trisaccharide provides two potential frame-shifted binding modes.

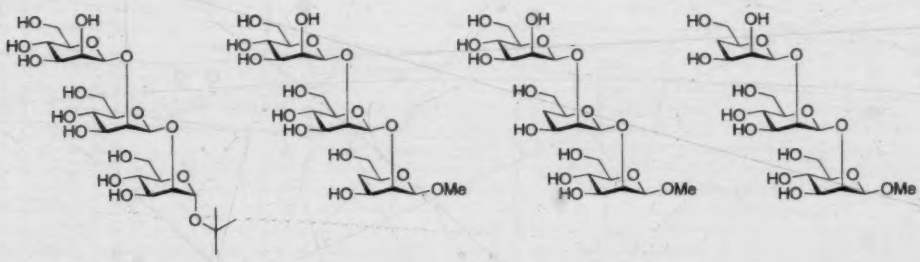
STD-NMR was used to analyze the binding of trisaccharide **2** to mAb C3.1. Unfortunately, despite extensive efforts an optimized pulse sequence was not realized and inadvertent ligand irradiation was observed in the control spectrum. However, lower ligand intensities in the control compared to the corresponding STD-NMR spectrum indicated contribution from saturation transfer. The STD spectrum was corrected by subtraction of the control to provide an epitope map. It appears that all three residues of the trisaccharide make contact with the protein and with H-2 of the terminal reducing residue receiving the greatest amount of magnetization transfer from the protein. Due to poor signal to noise and extensive signal overlap a complete epitope map was not obtained. The STD-NMR does not unambiguously distinguish either of the two frame shifted structures.

Repeating the analysis on trisaccharide **2** using a cryoprobe for better sensitivity and at higher field strength (i.e. 800 MHz) for greater signal dispersion is recommended. This should provide a more complete epitope map. Additional, STD-NMR experiments should include analysis of disaccharide **1** binding to mAb C3.1. This study will

complement the ELISA inhibition data and further define the continuous contact surface; however, it will not clarify the issue of frame shift that can occur for β-mannan structures larger than a disaccharide.

More quantitative evaluation of the synthetic analogues presented in this thesis is prudent. Attempts to generate a Fab fragment of mAb C3.1 are on-going for evaluation of ligand affinities using nano-ES-FT-ICR MS. Alternatively, the analogues could be screened by SPR using the full IgG. These experiments would provide data concerning the kinetics of binding. Determining the k_{off}, especially for the native di- and trisaccharides with mAb C3.1, provides important information for optimizing STD-NMR. Furthermore, this panel of synthetic analogues is a valuable tool for studying the epitope recognized by additional protective antibodies which may come available from immunization.

At this time, it is not possible to extrapolate to the trisaccharide epitope the continuous contact surface recognized by the antibody. The preparation and biological evaluation of additional synthetic trisaccharide analogues could be helpful in this regard:



There is the possibility that trisaccharide **2** binds in two different frame-shifted modes not distinguished by ELISA or STD-NMR. The native structure that elicited the antibody is predicted to be a trisaccharide which is attached through an α-phosphate

linkage to the remainder of the mannan complex. Considering this possibility, trisaccharide **2** having a β-linkage to the methyl aglycone may not be the most appropriate model. A trisaccharide having an α-linkage to a *t*-butyl aglycone may more closely mimic the native structure. Though the *t*-butyl does not provide the same electronics as the natural phosphodiester it could provide comparable sterics which may prevent shifting of the trisaccharide within the binding pocket. Lastly, deoxygenation at the C-4 and C-4′ positions both gave inactive compounds. Inhibition data from trisaccharides mono-deoxygenated at C-4, C-4′ and C-4″ should also provide a means to locate the residues important for binding.

Chapter Six

Experimental

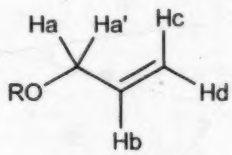
General synthesis and purification:

All chemical reagents were of analytical grade and used as obtained from Sigma-Aldrich unless indicated. Solvents used in water sensitive reactions was collected from a PURESOLV solvent purification system; except for DMSO which was distilled under vacuum and collected over 4 Å molecular sieves. Unless otherwise noted, reactions were carried out at room temperature and water-sensitive reactions were performed under an atmosphere of argon. Molecular sieves were flame dried and then allowed to cool to room temperature under argon before use. Solvents were removed under vacuum at between 20 and 40 °C (bath temperature). Analytical thin layer chromatography (TLC) was performed on silica gel 60-F_{254} (Merck). Plates were visualized under UV light, and/or by treatment with 5% sulfuric acid in ethanol followed by heating. Medium pressure chromatography was conducted using silica gel (230-400 mesh, Silicycle, Montreal) at flow rates between 5-10 mL min^{-1}. Following deprotection final compounds were passed through an Alltech Carbograph filter and then lyophilized.

Analytical procedures:

^{1}H NMR spectra were recorded on Varion INOVA 500 or 600 MHz spectrometers and ^{13}C NMR was recorded at 125 MHz. ^{1}H and ^{13}C NMR chemical shifts, reported in δ (ppm), were referenced to internal residual protonated solvent signals or to external acetone (0.1% ext. acetone @ 2.225 ppm) in the case of D_2O. ^{1}H and ^{13}C NMR assignments were made with the assistance of COSY, HSQC, HMBC and TOCSY

spectra where required. Values of $^{2}J_{H,H}$ and $^{3}J_{H,H}$ for carbohydrate resonances of defined multiplicity are indicated in Hz. Where these values have been omitted, the coupling constants were found to be in accord with the value observed for the corresponding coupling resonance and are only reported once. ^{1}H and ^{13}C chemical shifts are reported to one hundredth and one tenth of a ppm respectively except in some cases where additional figures are provided to distinguish closely resonating signals. A labeling scheme for hydrogen atoms of the allyl protecting groups is also provided.



Electrospray ionization mass spectra were recorded on a Micromass Zabspec TOF-mass spectrometer by analytical services in this department. For high resolution mass determination, spectra were obtained by voltage scan over a narrow range at a resolution of approximately 10^{4}. Optical rotations were determined with a Perkin-Elmer model 241 polarimeter at 22±2 °C using the sodium D-line and are reported in units of deg.·mL·g^{-1}·dm^{-1}. Elemental analysis was performed by the analytical services facility in this department.

Purification of monoclonal antibody C3.1:

Monoclonal antibody, mAb C3.1 (IgG3) was produced as a concentrated tissue culture supernatant and diluted ~1:20000 for ELISA measurements as previously described.

Oligosaccharide Inhibition of Enzyme linked immunosorbent assay:

C. albicans (1→2)-β-D-mannopyranoside trisaccharide-BSA conjugate in PBS (5 μg/mL) was used to coat 96-well ELISA plates (100 μL, 18 h at 4 °C). The plate was washed five times with PBST (PBS containing Tween 20, 0.05% v/v). Monoclonal antibody (C3.1) was mixed with inhibitor dissolved in PBST at concentrations between 1 mg/ml and 0.032 μg/mL; the resulting solutions were added to the coated microtiter plate in triplicate and incubated at room temperature for 2 h. The plate was washed five times with PBST. Goat anti-mouse IgG antibody conjugated to horseradish peroxidase (diluted 1:2000, Kirkegaard & Perry Laboratories) in PBST (100 μL) was added and incubated for 1 h at room temperature. The plate was washed five times with PBST. 3,3′,5,5′-tetramethylbenzidine (100 μL, Kirkegaard & Perry Laboratories) was added and after 20 minutes the color reaction was stopped by the addition of 1 M H_3PO_4 (100 μL). Absorbance was read at 450 nm, and percent inhibition was calculated relative to wells containing antibody without inhibitor. ELISA was performed by Joanna Sadowska.

Preparation of C3.1 for STD NMR:

Saturation transfer difference NMR spectroscopy used samples (650 μL, 2.8 mg mL^{-1}) of purified C3.1 (165 KDa) monoclonal antibody in deuterated phosphate buffer (PBS-D). Antibody (17.1 mg) in protonated buffer was lyophilized then redissolved in PBS-D (20 mL). The antibody PBS-D solution was concentrated to 4 mL using a Centricon filter. Deuterated phosphate buffer was prepared by lyophilizing PBS (0.10 mM PBS, 0.15 M NaCl) from D_2O three times.

Saturation Transfer Difference NMR:

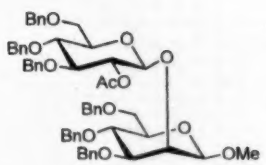
STD-NMR measurements were made on a Varian Inova 600 MHz spectrometer equipped with an inverse triple resonance probe at a temperature of 300 K. The pulse sequence followed that of Mayer and Meyer and used Watergate W5 for HOD suppression. The delay d3 was set to 1700 μs for optimal water suppression. One dimensional STD-NMR spectra were generated by subtraction of data acquired from the on-resonance (-1 ppm) and the off-resonance (35 ppm) pulses. The excitation of the protein consisted of 20 selective EBURP shaped pulses, each being 50 ms in length. The pulses were spaced 1 ms apart, resulting in 1.02 s of total protein irradiation. The number of transients was 14K for STD-NMR spectra. The reference spectra do not involve excitation of the protein and thus, the selective on-resonance pulse was set equal to the off-resonance pulse (35 ppm). The reference experiment was run for 7K with spectral subtraction removed by setting the difference flag to no. All spectra were calibrated to the HOD signal at 4.8 ppm.

The STD amplification factor was calculated using the following equation:

$$\eta = (I_o - I_{sat}/I_o) \times \text{ligand excess}$$

where $(I_o - I_{sat})$ is the peak height in the 1D STD NMR spectrum and I_o is the peak height of the corresponding signal in the reference spectrum. Ligand excess was 100-fold with respect to concentration of antibody (50-fold excess in terms of binding sites).

Compounds:

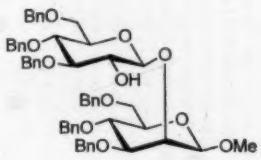


22

Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (22)

Methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside[183] **20** (390 mg, 0.84 mmol) and 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate **21**[175] (487 mg, 0.76 mmol) were combined in a flame dried flask (25 mL) and stored under vacuum for 2 h. The solids were dissolved in dry CH_2Cl_2 (10 mL), activated 4 Å molecular sieves were added and the mixture was cooled to 0 °C (ice-water bath) under argon. After stirring for 30 minutes, TMSOTf (5 μL) was added and the reaction was stirred for 1 hour at 0 °C. The reaction was neutralized with Et_3N, filtered through celite then concentrated under reduced pressure. Purification of the product by chromatography (7:3, hexanes-EtOAc) yielded **22** (678 mg, 95%); R_f 0.61 (1:1, hexanes-EtOAc); $[\alpha]_D$ -22 (*c* 0.36, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.19-7.38 (m, 30 H, ArH), 5.12 (dd, 1 H, $J_{1',2'}$ 8.1, $J_{2',3'}$ 9.7 Hz, H-2′), 4.94 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.89 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.83 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.82 (d, 1 H, H-1′), 4.79 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.76 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.57 (s, 2 H, PhCH_2O), 4.56 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.52 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.46-4.50 (m, 3 H, ArH), 4.24 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 4.22 (s, 1 H, H-1), 3.79 (dd, 1 H, $J_{5,6}$ 1.8, J_{gem} 10.7 Hz, H-6a), 3.77 (dd, 1 H, $J_{2',3'}$ 9.5, $J_{3',4'}$ 8.5 Hz, H-3′), 3.74 (dd, 1 H, $J_{5',6'}$ 1.5, J_{gem} 10.7, H-6a′), 3.65

(dd, 1 H, $J_{5',6'}$ 5.6, J_{gem} 10.6 Hz, H-6b'), 3.63 (dd, 1 H, $J_{5,6}$ 7.1, J_{gem} 10.8 Hz, H-6b), 3.58-3.62 (m, 3 H, H-4', H-5', H-4), 3.49 (s, 3 H, CH_3O), 3.48 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.2 Hz, H-3), 3.44 (ddd, 1 H, $J_{4,5}$ 9.5, $J_{5,6}$ 1.9, 7.1 Hz, H-5), 1.97 (s, 3 H, CH_3C(O)O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8 (*C*=O), 138.6 (Ar), 138.5(2) (Ar), 138.4(9) (Ar), 138.2 (Ar), 138.1 (Ar), 138.0 (Ar), 128.4 (Ar), 128.3(4) (Ar), 128.2(8) (Ar), 128.2(6) (Ar), 128.2 (Ar), 128.1(0) (Ar), 128.0(5) (Ar), 127.8 (Ar), 127.7(3) (Ar), 127.7(2) (Ar), 127.6(6) (Ar), 127.5(8) (Ar), 127.5(2) (Ar), 127.4(8) (Ar), 101.7 ($^1J_{C\text{-}1,H\text{-}1}$ 153.2 Hz, C-1 β), 101.2 ($^1J_{C\text{-}1',H\text{-}1'}$ 164.1 Hz, C-1' β), 83.1 (C-3'), 80.0 (C-3), 78.1 (C-4'/C-4), 75.6 (C-5), 75.2, 75.0, 74.8(2), 74.8(0), 74.7 (C-4'/C-4, C-5', Ph*C*H_2O×3), 73.6, 73.5, 73.2 (C-2', Ph*C*H_2O×2), 72.3 (C-2), 70.4, 69.9, 69.8 (C-6', C-6, Ph*C*H_2O), 56.7 (*C*H_3O), 21.1 (*C*H_3C(O)O); ESI HRMS Calc'd. for $C_{57}H_{62}O_{12}Na$ 961.4134. Found 961.4132.

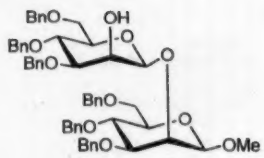


23

Methyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (23)

Disaccharide **22** (1.27 g, 1.36 mmol) was dissolved in a mixture of CH_2Cl_2 (10 mL) and methanol (10 mL). A solution of 0.5 M MeONa/MeOH (2 mL) was added and the reaction stirred at room temperature. After 2 h, the reaction was neutralized with Amberlite IR-120 (H^+) resin and filtered. The filtrate was concentrated under reduced pressure and the residue chromatographed over silica gel (7:3, hexanes-EtOAc) to give **23**

(1.19 g, 98%) as a white solid; R_f 0.47 (1:1, hexanes-EtOAc); $[\alpha]_D$ -39 (*c* 0.64, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18-7.34 (m, 30 H, ArH), 5.07 (d, 1 H, J_{gem} 11.4 Hz, PhCH_2O), 4.93 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.90 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.87 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.80 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.64 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1′), 4.64 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.56 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.56 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.53 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.49 (d, 1 H, J_{gem} 10.7 Hz, PhCH_2O), 4.49 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.45 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.29 (s, 1 H, H-1), 4.24 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 3.90 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4), 3.78 (dd, 1 H, $J_{5,6}$ 2.2, J_{gem} 11.0 Hz, H-6a), 3.70-3.76 (m, 3 H, H-2′, H-6a′, H-6b), 3.68 (dd, 1 H, $J_{2',3'} \approx J_{3',4'}$ 9.0 Hz, H-3′), 3.64 (dd, 1 H, $J_{5',6'}$ 5.7, J_{gem} 10.4 Hz, H-6b′), 3.51-3.59 (m, 6 H, H-4′, H-5′, H-3, CH_3O), 3.42 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6}$ 2.0, 4.8 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.1 (Ar), 138.4 (Ar), 138.2(9) (Ar), 138.2(5) (Ar), 138.1(3) (Ar), 138.0(6) (Ar), 128.4 (Ar), 128.3(2) (Ar), 128.3(0) (Ar), 128.2(5) (Ar), 128.1 (Ar), 128.0(44) (Ar), 128.0(40) (Ar), 127.9(5) (Ar), 127.8 (Ar), 127.7(1) (Ar), 127.6(7) (Ar), 127.6(1) (Ar), 127.5(7) (Ar), 127.5(6) (Ar), 127.5 (Ar), 127.4 (Ar), 104.2 (C-1′), 101.6 (C-1), 85.2 (C-3′), 80.2, 77.1 (C-4′, C-3), 75.7 (C-5), 75.4 (C-2′), 75.2 (C-5′, PhCH_2O), 75.0, 74.7(4), 74.6(6), 74.4 (C-2, C-4, PhCH_2O×2), 73.4(4) (PhCH_2O), 73.4(0) (PhCH_2O), 70.4 (PhCH_2O), 69.7 (C-6′), 69.2 (C-6), 57.1 (CH_3O); ESI HRMS Calc'd. for $C_{55}H_{60}O_{11}Na$ 919.4028. Found 919.4032. Anal. Calc'd. for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74. Found: C, 73.28; H, 6.81.

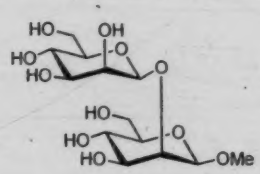


24

Methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (24)

Disaccharide **23** (1.28 g, 1.43 mmol) was dissolved in a mixture of Me_2SO (20 mL) and acetic anhydride (10 mL) then stirred overnight at room temperature. The mixture was concentrated under reduced pressure then the residue was redissolved in dry THF and cooled to -78 °C under argon. A solution of 1.0 M L-Selectride® (5.7 mL, 5.7 mmol) in THF was added dropwise and the reaction was stirred at -78 °C for 30 minutes. The reaction was quenched with methanol, diluted with CH_2Cl_2 then washed with 10% aqueous H_2O_2 solution, 1M aqueous NaOH, distilled water then brine. The organic phase was dried (Na_2SO_4) then concentrated under reduced pressure. The product was purified by column chromatography over silica gel (7:3, hexanes-EtOAc) to give **24** (1.03 g, 80%) as a clear syrup; R_f 0.43 (1:1, hexanes-EtOAc); $[\alpha]_D$ -50 (*c* 0.73, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22-7.42 (m, 28 H, ArH), 7.17-7.15 (m, 2 H, ArH), 4.97 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.94 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.92 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1′), 4.88 (d, 1 H, J_{gem} 10.7 Hz, PhCH_2O), 4.85 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.65 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.64 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.55-4.57 (m, 2 H, PhCH_2O), 4.51 (d, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 4.47 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.42-4.48 (m, 3 H, PhCH_2O), 4.34 (d, 1 H, $J_{2',3'}$ 2.9 Hz, H-2′), 4.29 (s, 1 H, H-1), 3.91 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.4 Hz, H-4′), 3.76-3.82 (m, 3 H, H-6a′, H-4, H-6a), 3.73 (dd, 1 H, $J_{5,6}$ 5.5, J_{gem}

10.8 Hz, H-6b), 3.64 (dd, 1 H, $J_{5',6'}$ 6.0, J_{gem} 10.4 Hz, H-6b'), 3.58 (dd, 1 H, $J_{2',3'}$ 2.9, $J_{3',4'}$ 9.0 Hz, H-3'), 3.56 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 9.3 Hz, H-3), 3.49-3.53 (m, 4 H, H-5', CH_3O), 3.43 (ddd, 1 H, $J_{4,5}$ 9.9, $J_{5,6}$ 2.1, 5.4 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5 (Ar), 138.4 (Ar), 138.3 (Ar), 138.1 (Ar), 128.4 (Ar), 128.3(4) (Ar), 128.3(2) (Ar), 128.3(1) (Ar), 128.2(9) (Ar), 128.2(7) (Ar), 128.2 (Ar), 128.1(3) (Ar), 128.0(6) (Ar), 127.9 (Ar), 127.7 (Ar), 127.6(2) (Ar), 127.5(9) (Ar), 127.5(4) (Ar), 127.5(3) (Ar), 102.1 (C-1), 99.1 (C-1'), 81.4 (C-3'), 80.3 (C-3), 75.6 (C-5), 75.1 (PhCH_2O×2), 75.0 (C-5'), 74.4 (C-4'), 74.1 (C-4), 73.5 (PhCH_2O), 73.4 (PhCH_2O), 70.7 (PhCH_2O), 70.3 (C-2), 70.1, 70.0 (C-6', PhCH_2O), 69.4 (C-6), 67.7 (C-2'), 57.2 (CH_3O); ESI HRMS Calc'd. for $C_{55}H_{60}O_{11}Na$ 919.4028. Found 919.4029.

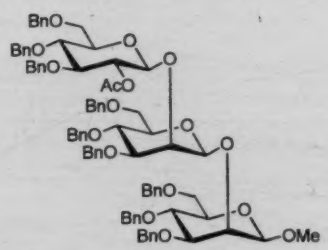


1

Methyl β-D-mannopyranosyl (1→2) β-D-mannopyranoside (1)

Disaccharide **24** (48 mg, 0.054 mmol) was dissolved in CH_2Cl_2 (5 mL) and methanol (5 mL). 10% Pd/C (50 mg) was added and the reaction was successively evacuated and purged with H_2 (g). The mixture was stirred overnight under a H_2 atmosphere. The catalyst was separated by filtration through a Whatman membrane (0.45 μm, PVDF) and the filtrate was concentrated under reduced pressure. The residue was redissolved in H_2O, passed through an Alltech Carbograph filter and then lyophilized to yield **1** (19 mg, 82%) as a clear glass; R_f 0.20 (6:3.5:0.5, CH_2Cl_2-MeOH-H_2O); $[\alpha]_D$ -

40 (*c* 0.31, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 4.81 (s, 1 H, H-1′), 4.64 (s, 1 H, H-1), 4.25 (d, 1 H, $J_{1,2}$ 3.1 Hz, H-2), 4.11 (d, 1 H, $J_{1',2'}$ 3.3 Hz, H-2′), 3.94 (dd, 1 H, $J_{5,6}$ 2.1, J_{gem} 12.3 Hz, H-6a), 3.92 (dd, 1 H, $J_{5',6'}$ 2.1, J_{gem} 12.2 Hz, H-6a′), 3.74 (d, 1 H, $J_{5,6}$ 6.7, J_{gem} 12.3 Hz, H-6b), 3.72 (dd, 1 H, $J_{5',6'}$ 7.5, J_{gem} 12.3 Hz, H-6b′), 3.62-3.64 (m, 2 H, H-3, H-3′), 3.58 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.7 Hz, H-4), 3.55 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.7 Hz, H-4′), 3.54 (s, 3 H, CH_3O), 3.35-3.42 (m, 2 H, H-5, H-5′); ^{13}C NMR (125 MHz, D_2O) δ 102.2 ($^1J_{C\text{-}1,H\text{-}1}$ 161.2 Hz, C-1 β), 101.4 ($^1J_{C\text{-}1',H\text{-}1'}$ 162.7 Hz, C-1′ β), 78.7 (C-2), 77.3, 77.2 (C-5, C-5′), 73.7, 73.2 (C-3, C-3′), 71.2 (C-2′), 68.2, 67.7 (C-4, C-4′), 62.0, 61.8 (C-6, C-6′), 58.0 (*C*H_3O); ESI HRMS Calc'd. for $C_{13}H_{24}O_{11}Na$ 379.1211. Found 379.1208.

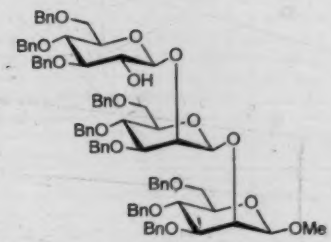


25

Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (25)

Compound **24** (205 mg, 0.23 mmol) and 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate[175] **21** (160 mg, 0.25 mmol) were reacted in CH_2Cl_2 (5 mL) with TMSOTf (5 μL,, 0.03 mmol) and processed as described for **1**. After chromatography with hexanes-EtOAc (3:2) trisaccharide **25** (244 mg, 78%) was obtained as a white solid; R_f 0.57 (1:1, hexanes-EtOAc); $[\alpha]_D$ +28 (*c* 0.17, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.07-7.39 (m, 45 H, ArH), 5.25 (d, 1 H, $J_{1'',2''}$ 8.2 Hz, H-1″), 5.19 (dd, 1

H, $J_{1'',2''}$ 8.2, $J_{2'',3''}$ 9.3 Hz, H-2''), 4.97 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.96 (d, 1 H, J_{gem} 10.5 Hz, PhCH_2O), 4.93 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.83 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.80 (d, 1 H, J_{gem} 11.1 Hz, PhCH_2O), 4.67-4.73 (m, 3 H, PhCH_2O×3), 4.62-4.64 (m, 2 H, H-1', PhCH_2O), 4.47-4.58 (m, 9 H, H-2', PhCH_2O×8), 4.43 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.23 (s, 1 H, H-1), 4.16 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.95 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.6 Hz, H-4), 3.89 (dd, 1 H, $J_{3'',4''}$ 8.6 Hz, H-3''), 3.78 (dd, 1 H, $J_{5',6'}$ 1.6, J_{gem} 10.5 Hz, H-6a'), 3.64-3.76 (m, 7 H, H-4'', H-5'', H-6a'', H-6b'', H-6b', H-6a, H-6b), 3.61 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.5 Hz, H-4'), 3.45-3.52 (m, 3 H, H-3', H-5', H-3), 3.44 (s, 3 H, CH_3O), 3.36 (ddd, 1 H, $J_{4,5}$ 9.9, $J_{5,6}$ 3.3, 3.3 Hz, H-5), 1.96 (s, 3 H, CH_3C(O)O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5 (*C*=O), 138.7 (Ar), 138.5 (Ar), 138.4 (Ar), 138.3(3) (Ar), 138.3(0) (Ar), 138.2(4) (Ar), 138.2(3) (Ar), 138.1 (Ar), 128.3(3) (Ar), 128.3(0) (Ar), 128.2(5) (Ar), 128.2(3) (Ar), 128.1(9) (Ar), 128.1(5) (Ar), 128.1(2) (Ar), 128.0(8) (Ar), 127.8(3) (Ar), 127.7(7) (Ar), 127.7(1) (Ar), 127.6(7) (Ar), 127.6(4) (Ar), 127.5(8) (Ar), 127.5 (Ar), 127.4(2) (Ar), 127.4(0) (Ar), 127.3 (Ar), 102.1 (C-1', C-1, $^1J_{C\text{-}1,H\text{-}1}$ 154.9, 157.4 Hz, both β), 101.0 (C-1'', $^1J_{C\text{-}1'',H\text{-}1''}$ 167.0 Hz, β), 83.6 (C-3''), 80.4 (C-3), 80.0 (C-3'), 78.3 (C-5''), 75.4, 75.3, 75.2, 74.9, 74.8, 74.7, 74.6(4), 74.5(6), 74.5(5) (C-4'', C-4', C-5', C-2, C-4, C-5, PhCH$_2$O×4), 73.4, 73.3, 73.2(3), 73.1(6) (C-2'', PhCH$_2$O×3), 71.8 (C-2'), 70.8 (C-6'), 69.9, 69.8, 69.5, 69.1 (C-6'', C-6, PhCH$_2$O×2), 56.9 (CH$_3$O), 21.2 (CH$_3$C(O)O); ESI HRMS Calc'd. for $C_{84}H_{90}O_{17}Na$ 1393.6070. Found 1393.6066. Anal. Calc'd. for $C_{84}H_{90}O_{17}$: C, 73.56; H, 6.61. Found: C, 73.27; H, 6.86.

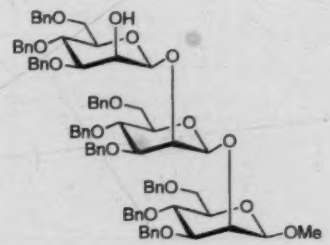


26

Methyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (26)

Trisaccharide **25** (115 mg, 0.084 mmol) was dissolved in a mixture of CH_2Cl_2 (2 ml) and methanol (2 mL) and treated with 0.5 M MeONa/MeOH (1 mL) then processed as described for **23**. Purification of the product by chromatography over silica gel (7:3, hexanes-EtOAc) gave **26** (102 mg, 92%); R_f 0.55 (1:1, hexanes-EtOAc); $[\alpha]_D$ -61 (*c* 0.54, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40-7.42 (m, 2 H, ArH), 7.36-7.38 (m, 4 H, ArH), 7.12-7.28 (m, 37 H, ArH), 7.00-7.02 (m, 2 H, ArH), 5.02 (d, 1 H, J_{gem} 11.6 Hz, PhCH_2O), 5.01 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 5.00 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.99 (d, 1 H, J_{gem} 12.7 Hz, PhCH_2O), 4.95 (d, 1 H, J_{gem} 11.1 Hz, PhCH_2O), 4.89 (s, 1 H, H-1′), 4.85 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.75 (d, 1 H, $J_{1'',2''}$ 7.7 Hz, H-1″), 4.68 (d, 1 H, J_{gem} 12. Hz, PhCH_2O), 4.60 (d, 1 H, J_{gem} 11.2 Hz, PhCH_2O), 4.60 (d, 1 H, J_{gem} 11.2 Hz, PhCH_2O), 4.53 (d, 1 H, J_{gem} 12.6 Hz, PhCH_2O), 4.51 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.46-4.49 (m, 5 H, H-2′, H-2, PhCH_2O×3), 4.43 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.42 (d, 1 H, J_{gem} 11.7 Hz, PhCH_2O), 4.37 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.36 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.28 (s, 1 H, H-1), 4.10 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.6 Hz, H-4), 3.82 (m, 5 H, H-2″, H-4′, H-6a′, H-6a, H-6b), 3.61-3.70 (m, 4 H, H-3″, H-6a″, H-6b″, H-6b′), 3.54-3.58 (m, 3 H, H-4″, H-5″, H-3′), 3.47-3.52 (m, 5 H, H-5′, H-3, CH_3O),

3.39 (ddd, 1 H, *J* 3.4, 3.4, 9.9 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.1 (Ar), 138.6(1) (Ar), 138.6(0) (Ar), 138.3(9) (Ar), 138.3(8) (Ar), 138.1(0) (Ar), 138.0(8) (Ar), 138.0 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2(3) (Ar), 128.2(0) (Ar), 128.1(8) (Ar), 128.1(2) (Ar), 128.0(7) (Ar), 128.0(4) (Ar), 127.9(6) (Ar), 127.9 (Ar), 127.8(0) (Ar), 127.7(8) (Ar), 127.7 (Ar), 127.6(1) (Ar), 127.5(6) (Ar), 127.5(1) (Ar), 127.4(6) (Ar), 127.3 (Ar), 127.1 (Ar), 105.2 ($^1J_{C\text{-}1'',H\text{-}1''}$ 162.9 Hz, C-1″ β), 102.2 ($^1J_{C\text{-}1,H\text{-}1}$ 154.8 Hz, C-1 β), 99.7 ($^1J_{C\text{-}1',H\text{-}1'}$ 162.2 Hz, C-1′ β), 86.7, 80.1, 80.0, 77.1, 75.5, 75.4(3), 75.3(5), 75.1(3), 75.1(1), 74.9, 74.8, 74.7, 74.6(4), 74.5(6), 74.0, 73.6, 73.4, 73.2, 70.6, 70.3, 70.1, 69.6(8), 69.6(5), 69.4, 57.4 (CH_3O); ESI HRMS Calc'd. for $C_{82}H_{88}O_{16}Na$ 1351.5965. Found 1351.5961; Anal. Calc'd. for $C_{35}H_{36}O_8$: C,74.08; H, 6.67. Found: C, 73.79; H, 6.61.



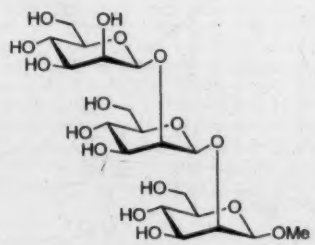
27

Methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (27)

Methyl trisaccharide **26** (250.4 mg, 0.19 mmol) was dissolved in a mixture of Me_2SO (5 mL) and acetic anhydride (2.5 mL) then processed as described for **24**. The oxidation product was redissolved in dry THF, cooled to -78 °C under an atmosphere of argon and reacted with L-Selectride® (1 M) in THF (790 μL, 0.79 mmol). The reaction mixture was diluted with CH_2Cl_2 then washed with 10% aqueous H_2O_2, 1 M aqueous

NaOH, distilled water then brine. The organic phase was dried (Na_2SO_4) then concentrated under reduced pressure. Purification of the product by chromatography (2:1, hexanes-EtOAc) gave **27** (191.2 mg, 77%) as a clear syrup; R_f 0.46 (1:1, hexanes-EtOAc); $[\alpha]_D$ -68 (*c* 1.22, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46-7.48 (m, 2 H, ArH), 7.39-7.41 (m, 2 H, ArH), 7.04-7.31 (m, 39 H, ArH), 6.94-6.96 (m, 2 H, ArH), 5.15 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1″), 5.06 (s, 1 H, H-1′), 4.98 (d, 1 H, J_{gem} 11.8 Hz, PhCH_2O), 4.97 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.94 (d, 1 H, J_{gem} 10.1 Hz, PhCH_2O), 4.85 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.77 (d, 1 H, J_{gem} 10.3 Hz, PhCH_2O), 4.70 (d, 1 H, $J_{2',3'}$ 3.2 Hz, H-2′), 4.64 (d, 1 H, J_{gem} 12.3 Hz, PhCH_2O), 4.61 (d, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 4.55 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.46-4.51 (m, 6 H, PhCH_2O×6), 4.43 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.40 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.33 (d, 1 H, J_{gem} 10.0 Hz, PhCH_2O), 4.31 (s, 1 H, H-1), 4.29 (d, 1 H, H-2″), 4.22 (d, 1 H, J_{gem} 10.3 Hz, PhCH_2O), 4.20 (d, 1 H, J_{gem} 11.8 Hz, PhCH_2O), 4.02 (d, 1 H, J_{gem} 11.7 Hz, PhCH_2O), 3.89 (d, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.3 Hz, H-4′), 3.87 (dd, 1 H, $J_{3'',4''} \approx J_{4'',5''}$ 9.3 Hz, H-4″), 3.79 (dd, 1 H, $J_{5',6'}$ 1.8, J_{gem} 10.6 Hz, H-6a′), 3.60-3.73 (m, 7 H, H-6a″, H-6b″, H-6b′, H-3′, H-6a, H-6b, H-4), 3.57 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 9.3 Hz, H-3), 3.49-3.55 (m, 2 H, H-5″, H-5′), 3.49 (s, 3 H, CH_3O), 3.47 (dd, 1 H, $J_{2'',3''}$ 3.3, $J_{3'',4''}$ 9.2 Hz, H-3″), 3.41 (ddd, 1 H, $J_{4,5}$ 9.4, $J_{5,6}$ 2.4, 4.3 Hz, H-5), 2.55 (bs, 1 H, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.6(1) (Ar), 138.5(9) (Ar), 138.4 (Ar), 138.3 (Ar), 138.1(9) (Ar), 138.1(8) (Ar), 138.1(3) (Ar), 138.0 (Ar), 137.9 (Ar), 129.1 (Ar), 128.4 (Ar), 128.3(4) (Ar), 128.3(1) (Ar), 128.2(4) (Ar), 128.2(0) (Ar), 128.1(9) (Ar), 128.1 (Ar), 128.0(3) (Ar), 128.0(1) (Ar), 127.9(8) (Ar), 127.8(6) (Ar), 127.8 (Ar), 127.7(1) (Ar), 127.6(6) (Ar), 127.6(2) (Ar), 127.5(1) (Ar), 127.4(9) (Ar), 127.3(8) (Ar), 127.3(6) (Ar), 127.3(4) (Ar), 127.3(1) (Ar), 127.1 (Ar), 102.7 ($^1J_{C\text{-}1,H\text{-}1}$ 154.1 Hz, C-1 β), 100.0

($^1J_{C\text{-}1',H\text{-}1'}$ 162.1 Hz, C-1′ β), 99.9 ($^1J_{C\text{-}1'',H\text{-}1''}$ 163.6 Hz, C-1″ β), 83.1 (C-3″), 80.4, 80.2, 75.4, 75.2, 75.1, 75.0, 74.8, 74.7, 74.4, 73.5, 73.4, 73.3, 71.2, 70.2, 70.1, 69.9(0), 69.8(6), 69.5, 69.1, 69.0, 67.3, 57.3 (CH_3O); ESI HRMS Calc'd. for $C_{82}H_{88}O_{16}Na$ 1351.5965. Found 1351.5967.

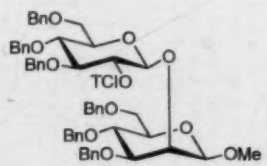


2

Methyl β-D-mannopyranosyl (1→2) β-D-mannopyranosyl (1→2) β-D-mannopyranoside (2)

Compound **27** (50.0 mg, 0.038 mmol) was dissolved in CH_2Cl_2 (5 mL)-methanol (5 mL) and stirred with 10% Pd/C (50 mg) under an H_2 atmosphere then processed as described for **1**. Filtration then lyophilization gave **2** (16.7 mg, 85%) as a clear glass; R_f 0.09 (5:4.5:0.5, CH_2Cl_2-MeOH-H_2O); $[\alpha]_D$ -74 (*c* 0.28, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 4.92 (s, 1 H, H-1″), 4.89 (s, 1 H, H-1′), 4.63 (s, 1 H, H-1), 4.35 (d, 1 H, $J_{2',3'}$ 3.3 Hz, H-2′), 4.23 (d, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 4.14 (d, 1 H, $J_{2'',3''}$ 3.3 Hz, H-2″), 3.94 (dd, 1 H, $J_{5,6}$ 1.3, J_{gem} 11.8 Hz, H-6a), 3.92 (dd, 1 H, $J_{5',6'}$ 2.0, J_{gem} 12.4 Hz, H-6a′), 3.92 (dd, 1 H, $J_{5'',6''}$ 1.5, J_{gem} 11.2 Hz, H-6a″), 3.75 (dd, 1 H, $J_{5',6'}$ 6.0, J_{gem} 12.5 Hz, H-6b′), 3.73 (dd, 1 H, $J_{5'',6''}$ 6.9, J_{gem} 12.0 Hz, H-6b″), 3.72 (dd, 1 H, $J_{5,6}$ 5.5, J_{gem} 12.3 Hz, H-6b), 3.67 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 9.9 Hz, H-3), 3.65 (dd, 1 H, $J_{2',3'}$ 3.2, $J_{3',4'}$ 10.1 Hz, H-3′), 3.62 (dd, 1 H, $J_{2'',3''}$ 3.2, $J_{3'',4''}$ 9.9 Hz, H-3″), 3.59 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.6 Hz, H-4′), 3.57 (dd, 1 H, $J_{3'',4''} \approx$

$J_{4'',5''}$ 9.3 Hz, H-4''), 3.54 (s, 3 H, CH_3O), 3.49 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.7 Hz, H-4), 3.38 (ddd, 1 H, H-5), 3.38 (ddd, 1 H, H-5''), 3.37 (ddd, 1 H, H-5'); ^{13}C NMR (125 MHz, D_2O) δ 102.2 ($^1J_{C\text{-}1,H\text{-}1}$ 159.3 Hz, C-1 β), 101.7(2) ($^1J_{C\text{-}1'',H\text{-}1''}$ 163.0 Hz, C-1'' β), 101.6(7) ($^1J_{C\text{-}1',H\text{-}1'}$ 159.3 Hz, C-1' β), 79.2 (C-2), 79.0 (C-2'), 77.2 (×2), 77.1 (C-5'', C-5', C-5), 73.9 (C-3''), 7.31 (C-3'), 72.9 (C-3), 71.3 (C-2''), 68.3 (C-4), 67.9 (C-4'), 67.6 (C-4''), 62.0, 61.7, 61.6 (C-6'', C-6', C-6), 58.0 (CH_3O); ESI HRMS Calc'd. for $C_{19}H_{34}O_{16}Na$ 541.1739. Found 541.1742.

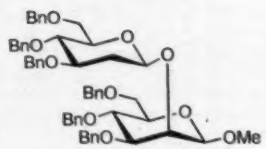


28

Methyl 3,4,6-tri-*O*-benzyl-2-*O*-thiocarbonylimidazole-β-D-glucopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (28)

Disaccharide **23** (200.0 mg, 0.22 mmol) was dissolved in dry toluene (4 mL). 1,1'-Thiocarbonyl diimidazole (60 mg, 0.33 mmol) was added and the reaction was refluxed under argon overnight. The reaction mixture was cooled then concentrated under reduced pressure. The oily residue was subjected to chromatography on silica gel (1:1, hexanes-EtOAc) to give **28** (199.7 mg, 89%); R_f 0.46 (1:4, hexanes-EtOAc); $[\alpha]_D$ -30 (*c* 0.26, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (s, 1 H, Im), 7.49 (s, 1 H, Im), 7.15-7.37 (m, 30 H, ArH), 6.93 (s, 1 H, Im), 5.85 (dd, 1 H, $J_{1',2'}$ 8.1, $J_{2',3'}$ 9.3 Hz, H-2'), 4.97 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.88 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.87 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.86 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O), 4.81 (d, 1 H, J_{gem} 11.7 Hz,

PhCH_2O), 4.62 (d, 1 H, J_{gem} 11.7 Hz, PhCH_2O), 4.61 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.52 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.52 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.48 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.46 (d, 1 H, J_{gem} 10.6 Hz, PhCH_2O), 4.34 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.28 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.20 (d, 1 H, $J_{2,3}$ 2.9 Hz, H-2), 4.06 (s, 1 H, H-1), 3.91 (dd, 1 H, $J_{2',3'} \approx J_{3',4'}$ 9.0 Hz, H-3'), 3.73-3.77 (m, 2 H, H-4', H-6a'), 3.69 (dd, 1 H, $J_{5',6'}$ 6.0, J_{gem} 10.6 Hz, H-6b'), 3.61-3.64 (m, 2 H, H-5', H-6a), 3.52 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.3 Hz, H-4), 3.41 (dd, 1 H, $J_{2,3}$ 2.9, $J_{3,4}$ 9.2 Hz, H-3), 3.23-3.31 (m, 5 H, H-5, H-6b, CH_3O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 182.5 (*C*=S), 138.5 (Ar), 138.3 (Ar), 138.0 (Ar), 137.8 (Ar), 137.7 (Ar), 137.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3(2) (Ar), 128.2(89) (Ar), 128.2(81) (Ar), 128.2 (Ar), 128.1(4) (Ar), 128.1(0) (Ar), 128.0(6) (Ar), 128.0 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.4 (Ar), 119.0 (Ar), 101.8 (C-1), 100.6 (C-1'), 82.1 (C-3'), 81.6 (C-2'), 79.8 (C-3), 78.4 (C-4'), 75.8 (C-5'), 75.2, 75.1, 75.0(1), 74.9(9) (C-5, PhCH_2O×3), 74.4 (C-4), 73.6 (PhCH_2O), 73.4 (PhCH_2O), 72.7 (C-2), 70.2, 70.1 (C-6, PhCH_2O), 69.4 (C-6'), 57.3 (CH_3O); ESI HRMS Calc'd. for $C_{59}H_{62}N_2O_{11}SNa$ 1029.3967. Found 1029.3970; Anal. Calc'd. for $C_{59}H_{62}N_2O_{11}S$: C, 70.36; H, 6.20; N, 2.78; S, 3.18. Found: C, 70.27; H, 6.21; N, 2.79; S, 3.04.

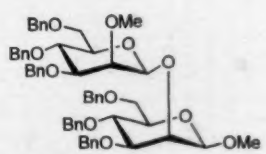


29

Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-β-D-*arabino*-hexopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (29)

Compound **28** (179.2 mg, 0.18 mmol) was dissolved in dry toluene (4 mL). Tributyltin hydride (120 μL, 0.45 mmol) and azobisisobutyronitrile (3 mg, 0.018 mmol) were added and the reaction was refluxed under argon. After 2 hours, the mixture was concentrated under reduced pressure. The brown oily residue was subjected to chromatography on silica gel (9:1, hexanes-EtOAc) to give **29** (98.4 mg, 63%); R_f 0.65 (1:1, hexanes-EtOAc); $[\alpha]_D$ -48 (*c* 0.72, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39-7.21 (m, 28 H, ArH), 7.18-7.16 (m, 2 H, ArH), 4.96 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.95 (d, 1 H, J_{gem} 10.5 Hz, PhCH_2O), 4.93 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.87 (dd, 1 H, $J_{1',2'}$ 1.8, 9.8 Hz, H-1′), 4.71 (d, 1 H, J_{gem} 11.6 Hz, PhCH_2O), 4.64 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.57 (d, 1 H, J_{gem} 11.1 Hz, PhCH_2O), 4.56 (d, 1 H, J_{gem} 11.7 Hz, PhCH_2O), 4.55 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.53 (d, 1 H, J_{gem} 11.8 Hz, PhCH_2O), 4.48-4.44 (m, 2 H, PhCH_2O), 4.46 (d, 1 H, J_{gem} 10.7 Hz, PhCH_2O), 4.38 (d, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 4.25 (s, 1 H, H-1), 3.84 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4), 3.79 (dd, 1 H, $J_{5,6}$ 2.2, J_{gem} 10.9 Hz, H-6a), 3.77 (dd, 1 H, $J_{5',6'}$ 1.9, J_{gem} 10.4 Hz, H-6a′), 3.76 (dd, 1 H, $J_{5,6}$ 5.2, J_{gem} 10.8 Hz, H-6b), 3.71 (ddd, 1 H, $J_{2',3'}$ 5.1, 11.6, $J_{3',4'}$ 8.5 Hz, H-3′), 3.65 (dd, 1 H, $J_{5',6'}$ 6.3, J_{gem} 10.4 Hz, H-6b′), 3.53-3.50 (m, 2 H, H-5′, H-3), 3.51 (s, 3 H, CH_3O), 3.46 (dd, 1 H, $J_{3',4'}$ 8.5, $J_{4',5'}$ 9.6 Hz, H-4′), 3.41 (ddd, 1 H, $J_{4,5}$ 9.7, J_{gem} 2.1, 5.2 Hz, H-5), 2.61 (ddd, 1 H, $J_{1',2'}$ 1.8, $J_{2',3'}$

4.8, J_{gem} 12.4 Hz, H-2eq'), 1.77 (ddd, 1 H, $J_{1',2'}$ 9.9, $J_{2',3'} \approx J_{gem}$ 11.9 Hz, H-2ax'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.6 (Ar), 138.5(4) (Ar), 138.4(8) (Ar), 138.4 (Ar), 138.3 (Ar), 138.2 (Ar), 128.4 (Ar), 128.3(3) (Ar), 128.3(0) (Ar), 128.2(8) (Ar), 128.2(6) (Ar), 128.2(3) (Ar), 128.2(2) (Ar), 128.1 (Ar), 128.0 (Ar), 127.8 (Ar), 127.7(1) (Ar), 127.6(6) (Ar), 127.5(9) (Ar), 127.4(9) (Ar), 127.4(6) (Ar), 127.4(5) (Ar), 102.3 ($^1J_{C\text{-}1,H\text{-}1}$ 153.1 Hz, C-1 β), 100.4 ($^1J_{C\text{-}1',H\text{-}1'}$ 163.0 Hz, C-1′ β), 80.0 (C-3/C-5′), 79.8 (C-3′), 78.1 (C-4′), 75.7 (C-5), 75.1 (Ph*C*H_2O), 74.9, 74.8 (Ph*C*H_2O, C-3/C-5′), 74.3 (C-4), 73.3(8) (Ph*C*H_2O), 73.3(5) (Ph*C*H_2O), 71.8 (C-2), 70.9 (Ph*C*H_2O), 70.4 (C-6′), 69.8, 69.5 (C-6, Ph*C*H_2O), 57.2 (*C*H_3O), 36.2 (C-2′); ESI HRMS Calc'd. for $C_{55}H_{60}O_{10}Na$ 903.4079. Found 903.4080.

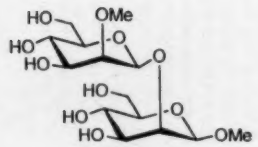


30

Methyl 3,4,6-tri-*O*-benzyl-2-*O*-methyl-β-D-mannopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (30)

Compound **24** (200 mg, 0.22 mmol) was dissolved in dry DMF (2 mL) and the solution was cooled to 0 °C (ice-water bath). Methyl iodide (155 μL, 0.67 mmol) and sodium hydride (16 mg, 0.67 mmol) were added and the reaction was kept at 0 °C. After two hours, the reaction was quenched with methanol and concentrated under reduced pressure. The residue was subjected to column chromatography over silica gel (7:3, hexanes-EtOAc) to give **30** (185 mg, 91%) as a clear syrup; R_f 0.48 (1:1, hexanes-

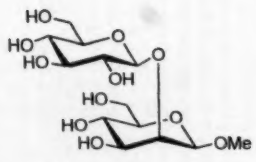
EtOAc); $[\alpha]_D$ -49 (*c* 1.13, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.43-7.44 (m, 2 H, ArH), 7.39-7.40 (m, 2 H, ArH), 7.20-7.34 (m, 24 H, ArH), 7.15-7.17 (m, 2 H, ArH), 4.99 (d, 1 H, J_{gem} 11.7 Hz, PhCH_2O), 4.94 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.89 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.84 (s, 1 H, H-1′), 4.77 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.60-4.64 (m, 2 H, PhCH_2O), 4.53 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.52 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.48 (d, 1 H, J_{gem} 11.8 Hz, PhCH_2O), 4.46 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.42 (m, 2 H, PhCH_2O), 4.39 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 4.26 (s, 1 H, H-1), 3.91 (d, 1 H, $J_{2',3'}$ 3.2 Hz, H-2′), 3.82 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.6 Hz, H-4), 3.79 (dd, 1 H, $J_{5,6}$ 1.9, J_{gem} 10.7 Hz, H-6a), 3.72-3.75 (m, 2 H, H-4′, H-6a′), 3.71 (s, 3 H, CH_3O), 3.71 (dd, 1 H, $J_{5,6}$ 5.2, J_{gem} 10.6 Hz, H-6b), 3.63 (dd, 1 H, $J_{5',6'}$ 6.8, J_{gem} 10.4 Hz, H-6b′), 3.54 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.3 Hz, H-3), 3.52 (dd, 1 H, $J_{2',3'}$ 3.2, $J_{3',4'}$ 9.3 Hz, H-3′), 3.47-3.50 (m, 4 H, H-5′, CH_3O), 3.41 (ddd, 1 H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.6 (Ar), 138.5 (Ar), 138.4 (Ar), 138.3(4) (Ar), 138.3(0) (Ar), 138.2(Ar), 128.4 (Ar), 128.3(1) (Ar), 128.3(0) (Ar), 128.2(5) (Ar), 128.2 (Ar), 128.1 (Ar), 128.0 (Ar), 127.8 (Ar), 127.7(3) (Ar), 127.6(9) (Ar), 127.6 (Ar), 127.5(7) (Ar), 127.5(5) (Ar), 127.5(1) (Ar), 127.4 (Ar), 127.3 (Ar), 102.4 (C-1), 101.6 (C-1′), 81.8 (C-3), 79.8 (C-3′), 77 (C-2′) 75.4(4), 75.4(0), 75.2, 75.1, 75.0 (C-4′, C-5′, C-5, PhCH_2O×2), 74.0 (C-4), 73.4 (PhCH_2O), 73.3 (PhCH_2O), 71.6 (C-2), 71.1 (PhCH_2O), 70.4 (C-6′), 69.5, 69.4 (C-6, PhCH_2O), 61.4 (CH_3O), 57.3 (CH_3O); ESI HRMS Calc'd. for $C_{56}H_{62}O_{11}Na$ 933.4184. Found 933.4184; Anal. Calc'd. for $C_{56}H_{62}O_{11}$: C, 73.82; H, 6.86. Found: C, 73.76; H, 6.90.



4

Methyl 2-*O*-methyl-β-D-mannopyranosyl (1→2) β-D-mannopyranoside (4)

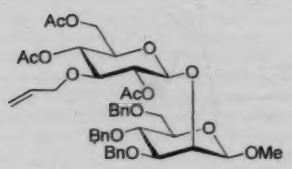
Compound **30** (50 mg, 0.055 mmol) was dissolved in CH_2Cl_2 (5 mL)-methanol (5 mL) and stirred with 10% Pd/C (50 mg) under an H_2 atmosphere then processed as described for **1**. Filtration then lyophilization gave **4** (20 mg, quant.) as a clear glass; R_f 0.28 (6:3.5:0.5, CH_2Cl_2-MeOH-H_2O); $[\alpha]_D$ -85 (*c* 0.70, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 4.86 (s, 1 H, H-1′), 4.64 (s, 1 H, H-1), 4.21 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 3.90-3.95 (m, 2 H, H-6a′, H-6b′), 3.83 (d. 1 H, $J_{2',3'}$ 3.4 Hz, H-2′), 3.71 (dd, 1 H, $J_{5,6}$ 6.5, J_{gem} 12.2 Hz, H-6b), 3.69 (dd, 1 H, $J_{5',6'}$ 7.1, J_{gem} 12.1 Hz, H-6b′), 3.63-3.65 (m, 2 H, H-3, H-3′), 3.59 (s, 3 H, C*H*$_3$O-C2′), 3.55 (m, 1 H, H-4), 3.55 (s, 3 H, C*H*$_3$O-C1), 3.45 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.8 Hz, H-4′), 3.40 (ddd, 1 H, $J_{4,5}$ 9.4, $J_{5,6}$ 1.8, 6.4 Hz, H-5), 3.32 (ddd, 1 H, $J_{5',6'}$ 1.8, 7.0 Hz, H-5′); ^{13}C NMR (125 MHz, D_2O) δ 102.3 (C-1, $^1J_{C\text{-}1,H\text{-}1}$ 159.8 Hz, β), 102.0 (C-1′, $^1J_{C\text{-}1',H\text{-}1'}$ 162.5 Hz, β), 81.3 (C-2′), 78.4 (C-2), 77.3, 77.2 (C-5, C-5′), 73.7, 73.1 (C-3, C-3′), 68.4, 68.1 (C-4, C-4′), 62.6 (*C*H$_3$OC-2′), 62.08, 61.98 (C-6, C-6′), 58.1 (*C*H$_3$OC-1); ESI HRMS Calc'd. for $C_{14}H_{26}O_{11}Na$ 393.1367. Found 393.1368.



5

Methyl β-D-glucopyranosyl (1→2) β-D-mannopyranoside (5)

Compound **23** (50.5 mg, 0.056 mmol) was dissolved in CH_2Cl_2 (5 mL)-methanol (5 mL) and stirred with 10% Pd/C (50 mg) under an H_2 atmosphere then processed as described for **1**. Filtration then lyophilization gave **5** (17 mg, 86%) as a clear glass; R_f 0.20 (6:3.5:0.5, CH_2Cl_2-MeOH-H_2O); $[\alpha]_D$ -59 (*c* 0.87, H_2O); ^{1}H NMR (500 MHz D_2O) δ 4.66 (s, 1 H, H-1), 4.58 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′) 4.23 (d, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 3.95 (dd, 1 H, $J_{5,6}$ 2.2, J_{gem} 12.4 Hz, H-6a), 3.91 (dd, 1 H, $J_{5',6'}$ 1.8, J_{gem} 12.2 Hz, H-6a′), 3.72-3.76 (m, 2 H, H-6b′, H-6b), 3.66 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.7 Hz, H-3), 3.53-3.57 (m, 4 H, H-4, C*H*$_3$O), 3.49 (dd, 1 H, $J_{2',3'} \approx J_{3',4'}$ 9.0 Hz, H-3′), 3.39-3.43 (m, 3 H, H-2′, H-5′, H-5), 3.37 (dd, 1 H, $J_{3',4'}$ 9.3, $J_{4',5'}$ 7.9 Hz, H-4′); ^{13}C NMR (125 MHz, D_2O) δ 104.2 (C-1′, $^1J_{C-1',H-1'}$ 165.0 Hz, β), 102.2 (C-1, $^1J_{C1,H1}$ 160.2 Hz, β), 79.0 (C-2), 77.4, 76.8, 76.3, 74.3 (C-4′/C-5′/C-5, C-2′, C-3′), 73.0 (C-3), 70.4 (C-4′/C-5′/C-5), 68.2 (C-4), 61.7, 61.5 (C-6′, C-6), 57.8 (*C*H$_3$O); ESI HRMS Calc'd. for $C_{13}H_{24}O_{11}Na$ 379.1211. Found 379.1204.

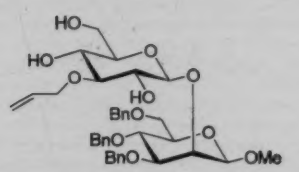


32

Methyl 2,4,6-tri-*O*-acetyl-3-*O*-allyl-β-D-glucopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (32)

Methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside[183] **20** (570.2 mg, 1.23 mmol) and 2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-glucopyranosyl trichloroacetimidate **31** (501.9 mg, 1.02 mmol) combined in a flame dried flask were kept under vacuum for 1 hour then dissolved in dry CH_2Cl_2 (10 mL). The mixture was cooled to -20 °C under argon and 4 Å molecular sieves were added. TMSOTf (19 μL) was added then the reaction was slowly warmed to room temperature. After 1 hour, the reaction was neutralized with Et_3N then concentrated under reduced pressure. Chromatography over silica gel (7:3, hexanes-EtOAc) gave **32** (437.8 mg, 54%) as a white solid; R_f 0.49 (1:1, hexanes-EtOAc); $[\alpha]_D$ -43 (*c* 0.46, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26-7.38 (m, 3 H, ArH), 7.18-7.20 (m, 2 H, ArH), 5.80 (dddd, 1 H, *J* 5.7, 5.7, 11.1, 17.2 Hz, H-b), 5.22 (dddd, 1 H, *J* 1.6, 1.6, 1.6, 17.2 Hz, H-c), 5.13 (dddd, 1 H, *J* 1.3, 1.3, 1.3, 10.4, H-d), 5.10 (dd, 1 H, $J_{1',2'}$ 8.1, $J_{2',3'}$ 9.7 Hz, H-2′), 5.07 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.4 Hz, H-4′), 4.91 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.86 (d, 1 H, H-1′), 4.83 (d, 1 H, J_{gem} 11.6 Hz, PhCH_2O), 4.57 (s, 2 H, PhCH_2O), 4.51 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.46 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.25 (s, 1 H, H-1), 4.19-4.21 (m, 3 H, H-6a′, H-6b′, H-2), 4.13 (dddd, 1 H, *J* 1.5, 1.5, 5.4, 12.8 Hz, H-a), 4.06 (dddd, 1 H, *J* 1.5, 1.5, 5.8, 12.7 Hz, H-a′), 3.79 (dd, 1 H, $J_{5,6}$ 1.7, J_{gem} 10.5 Hz, H-6a), 3.67 (dd, 1 H, $J_{2',3'} \approx J_{3',4'}$ 9.5 Hz, H-3′), 3.58-3.64 (m, 3 H, H-5′, H-4, H-

6b), 3.51 (s, 3 H, C*H*$_3$O), 3.49 (m, 1 H, H-3), 3.45 (ddd, 1 H, *J* 1.7, 7.1, 9.6 Hz, H-5), 2.09 (s, 3 H, C*H*$_3$C(O)O), 2.03 (s, 3 H, C*H*$_3$C(O)O), 2.01 (s, 3 H, C*H*$_3$C(O)O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7 (*C*=O), 169.42 (*C*=O), 169.36 (*C*=O), 138.43 (Ar), 138.41 (Ar), 138.0 (Ar), 134.6 (C=C), 128.33 (Ar), 128.32 (Ar), 128.29 (Ar), 128.1 (Ar), 128.0 (Ar), 127.7 (Ar), 127.64 (Ar), 127.56 (Ar), 127.5 (Ar), 116.6 (C=C), 101.5, 101.3 (C-1′/C-1), 80.4 (C-3), 79.6 (C-3′), 75.7 (C-5), 75.3 (Ph*C*H$_2$O), 74.7 (C-5′/C-4), 73.2 (Ph*C*H$_2$O), 72.7, 72.5, 72.2, 72.0 (C-2′, C-2, C-5′/C-4, CH$_2$-allyl), 70.33, 70.27 (C-6, Ph*C*H$_2$O), 69.7 (C-4′), 63.0 (C-6′), 56.7 (*C*H$_3$O), 21.0, 20.9, 20.8 (*C*H$_3$C(O)O×3); ESI HRMS Calc'd. for $C_{43}H_{52}O_{14}Na$ 815.3249. Found 815.3205.

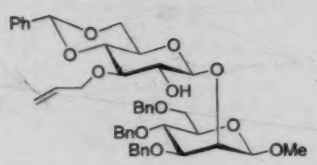


33

Methyl 3-*O*-allyl-β-D-glucopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (33)

Compound **32** (414.3 mg, 0.523 mmol) was dissolved in CH_2Cl_2 (5 mL) then 1.5 M MeONa/MeOH (2 mL) was added and the reaction stirred at room temperature. After 2 hours, TLC (9:1 CH_2Cl_2-MeOH) indicated the conversion was complete and the reaction was neutralized with Amberlite resin IR-120 (H^+), filtered then concentrated under reduced pressure. Chromatography over silica gel (9:1, CH_2Cl_2-MeOH) gave **33** (316.6 mg, 91%) as a white solid; R_f 0.47 (9:1, CH_2Cl_2-MeOH); $[\alpha]_D$ -46 (*c* 1.14, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27-7.40 (m, 13 H, ArH), 7.18-7.20 (m, 2 H, ArH), 5.98

(dddd, 1 H, *J* 5.5, 6.0, 10.4, 17.1 Hz, H-b), 5.31 (dddd, 1 H, *J* 1.7, 1.7, 1.7, 17.3 Hz, H-c), 5.19 (dddd, 1 H, *J* 1.2, 1.2, 1.2, 10.3 Hz, H-d), 4.90 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O) 4.81 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.64 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.64 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.63 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.55 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.53 (dddd, 1 H, *J* 1.5, 1.5, 5.5, 12.6 Hz, H-a), 4.51 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.31 (s, 1 H, H-1), 4.21 (dddd, 1 H, *J* 1.3, 1.3, 6.1, 12.8 Hz, H-a′), 4.16 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.90 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4), 3.87 (dd, 1 H, $J_{5,6}$ 3.7, J_{gem} 11.8 Hz, H-6a′), 3.72-3.79 (m, 3 H, H-6b′, H-6a, H-6b), 3.60 (dd, 1 H, $J_{1',2'}$ 7.9, $J_{2',3'}$ 9.1 Hz, H-2′), 3.54-3.58 (m, 2 H, H-4′, H-3), 3.54 (s, 3 H, CH_3O), 3.38-3.45 (m, 2 H, H-5′, H-5), 3.35 (dd, 1 H, $J_{2',3'} \approx J_{3',4'}$ 9.1 Hz, H-3′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.3 (Ar), 138.2 (Ar), 137.8 (Ar), 135.2 (CH_2=*C*H-CH_2), 128.5 (Ar), 128.4 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 117.0 (*C*H_2=CH-CH_2), 104.5 (C-1′), 101.4 (C-1), 83.9 (C-3′), 80.8 (C-4′/C-3), 75.7, 75.6, 75.4, 75.3, 74.8, 74.5 (C-2′, C-5′, C-2, C-4, C-5, Ph*C*H_2O), 73.5, 73.1 (Ph*C*H_2O, CH_2=CH-*C*H_2), 71.3 (Ph*C*H_2O), 70.1 (C-4′/C-3), 69.1 (C-6), 63.0 (C-6′), 57.1 (*C*H_3O); ESI HRMS Calc'd. for $C_{37}H_{46}O_{11}Na$ 689.2932. Found 689.2934.

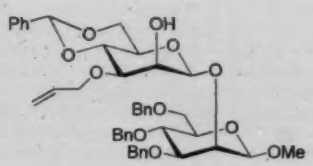


34

Methyl 3-*O*-allyl-4,6-*O*-benzylidene-β-D-glucopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (34)

Compound **33** (303.7 mg, 0.455 mmol) was dissolved in dry acetonitrile (5 mL). Benzaldehyde dimethyl acetal (107 μL, 0.713 mmol) was added followed by camphor sulfonic acid (5.5 mg). The reaction was kept under a slight vacuum on a rotary evaporator. After 1 hour, the reaction was neutralized with Et_3N then concentrated under reduced pressure. Chromatography over silica gel (3:2, hexanes-EtOAc) gave **34** (326.8 mg, 91%) as a white solid; R_f 0.25 (1:1, hexanes-EtOAc); $[\alpha]_D$ -40 (*c* 0.68, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.49-7.51 (m, 2 H, ArH), 7.27-7.40 (m, 16 H, ArH), 7.19-7.21 (m, 2 H, ArH), 5.99 (dddd, 1 H, *J* 5.7, 5.7, 11.3, 17.3 Hz, H-b), 5.54 (s, 1 H, PhC*H*O_2), 5.33 (dddd, 1 H, *J* 1.7, 1.7, 1.7, 17.1 Hz, H-c), 5.16 (dddd, 1 H, *J* 1.3, 1.3, 1.3, 10.4 Hz, H-d), 4.91 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.78 (d, 1 H, J_{gem} 11.7 Hz, PhCH_2O), 4.69 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.63 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.63 (d, 1 H, J_{gem} 11.6 Hz, PhCH_2O), 4.56 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.51 (d, 1 H, J_{gem} 10.7 Hz, PhCH_2O), 4.40 (dddd, 1 H, *J* 1.3, 1.3, 5.6, 12.8 Hz, H-a), 4.36 (dddd, 1 H, *J* 1.4, 1.4, 5.7, 12.9 Hz, H-a′), 4.33 (s, 1 H, H-1), 4.26 (dd, 1 H, $J_{5',6'}$ 5.1, J_{gem} 10.4 Hz, H-6eq′), 4.18 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 3.88 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4), 3.84 (dd, 1 H, $J_{5',6'} \approx J_{gem}$ 10.2 Hz, H-6ax′), 3.78 (dd, 1 H, $J_{5,6}$ 2.0, J_{gem} 11.0 Hz, H-6a), 3.74 (dd, 1 H, $J_{5,6}$ 5.1, J_{gem} 11.0 Hz, H-6b), 3.69-3.73 (m, 2 H, H-2′, H-4′), 3.63 (dd, 1 H, $J_{2',3'} \approx J_{3',4'}$ 8.9 Hz, H-3′),

3.54-3.56 (m, 5 H, H-3, C2-OH, CH_3O), 3.42-3.46 (m, 2 H, H-5′, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.3 (Ar), 138.2 (Ar), 137.9 (Ar), 137.4 (Ar), 135.4 (CH_2=*C*H-CH_2), 128.9 (Ar), 128.4 (Ar), 128.2 (Ar), 128.0 (Ar), 127.8 (Ar), 127.7(4) (Ar), 127.6(8) (Ar), 127.6 (Ar), 126.0 (Ar), 116.7 (*C*H_2=CH-CH_2), 105.4 (C-1′), 101.3 (C-1, Ph*C*HO_2), 80.9, 80.8 (C-2′/C-4′, C-3), 80.3 (C-3′), 76.3 (C-2), 75.8 (C-5′/C-5), 75.3 (C-2′/C-4′), 75.0 (Ph*C*H_2O), 74.5 (C-4), 73.5, 73.4 (Ph*C*H_2O, CH_2=CH-*C*H_2), 71.4 (Ph*C*H_2O), 69.1 (C-6′), 68.7 (C-6), 66.7 (C-5′/C-5), 57.1 (*C*H_3O); ESI HRMS Calc'd. for $C_{44}H_{50}O_{11}Na$ 777.3245. Found 777.3242; Anal. Calc'd. for $C_{44}H_{50}O_{11}$: C, 70.01; H, 6.68. Found: C, 70.15; H, 6.72.

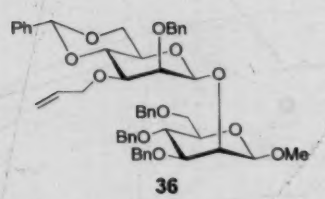


35

Methyl 3-*O*-allyl-4,6-*O*-benzylidene-β-D-mannopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (35)

Disaccharide **34** (313.8 mg, 0.42 mmol) was dissolved in a mixture of Me_2SO (2 mL) and acetic anhydride (1 mL) then processed as described for **24**. The oxidation product was redissolved in dry THF (5 mL), cooled to -78 °C under an atmosphere of argon and reacted with L-Selectride® (1 M) in THF (2 mL, 2.08 mmol). The reaction mixture was diluted with CH_2Cl_2 then washed with 10% aqueous H_2O_2, 1 M aqueous NaOH, distilled water then brine. The organic phase was dried (Na_2SO_4) then concentrated under reduced pressure. Purification of the product by chromatography (3:2, hexanes-EtOAc) gave **35** (212.6 mg, 68%) as a white solid; R_f 0.23 (1:1, hexanes-

EtOAc); $[\alpha]_D$ -71 (*c* 0.50, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.48-7.50 (m, 2 H, ArH), 7.25-7.41 (m, 16 H, ArH), 7.15-7.17 (m, 2 H, ArH), 5.96 (dddd, 1 H, *J* 5.7, 5.7, 10.4, 17.3 Hz, H-b), 5.56 (s, 1 H, PhC*H*O_2), 5.33 (dddd, 1 H, *J* 1.6, 1.6, 1.6, 17.2 Hz, H-c), 5.19 (dddd, 1 H, *J* 1.3, 1.3, 1.3, 10.4 Hz, H-d), 4.98 (d, 1 H, $J_{1',2'}$ 0.9 Hz, H-1′), 4.86 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.80 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.63 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.56 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.54 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.48 (d, 1 H, J_{gem} 10.7 Hz, PhCH_2O), 4.38 (d, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 4.28-4.33 (m, 4 H, H-2′, H-6a′, H-1, H-a), 4.25 (dddd, 1 H, *J* 1.4, 1.4, 5.9, 13.1 Hz, H-a′), 4.17 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.5 Hz, H-4′), 3.77-3.83 (m, 3 H, H-6b′, H-4, H-6a), 3.72 (dd, 1 H, $J_{5,6}$ 5.3, J_{gem} 10.8 Hz, H-6b), 3.61 (dd, 1 H, $J_{2',3'}$ 3.2, $J_{3',4'}$ 9.7 Hz, H-3′), 3.56 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 9.3 Hz, H-3), 3.53 (s, 3 H, CH_3O), 3.43 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6}$ 1.9, 5.3 Hz, H-5), 3.40 (ddd, 1 H, $J_{4',5'}$ 9.9, $J_{5',6'}$ 4.8, 9.9 Hz, H-5′), 2.86 (bs, 1 H, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.3 (Ar), 138.2 (Ar), 138.1 (Ar), 137.6 (Ar), 135.0 (H_2C=*C*H-CH_2), 128.9 (Ar), 128.3(55) (Ar), 128.3(51) (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 127.8(95) (Ar), 127.8(83) (Ar), 127.7(0) (Ar), 127.6(6) (Ar), 127.6 (Ar), 126.1 (Ar), 117.2 (H_2C=CH-CH_2), 101.8 (C-1), 101.6 (Ph*C*HO_2), 100.1 (C-1′), 80.7 (C-3), 78.2 (C-4′), 76.8 (C-3′), 75.6 (C-5), 75.2 (Ph*C*H_2O), 74.2 (C-4), 73.5 (Ph*C*H_2O), 71.7 (C-2), 71.4 (H_2C=CH-*C*H_2), 70.8 (Ph*C*H_2O), 69.4, 69.3 (C-2′, C-6), 68.7 (C-6′), 67.1 (C-5′), 57.2 (*C*H_3O); ESI HRMS Calc'd. for $C_{44}H_{50}O_{11}Na$ 777.3245. Found 777.3244; Anal. Calc'd. for $C_{44}H_{50}O_{11}$: C, 70.01; H, 6.68. Found: C, 69.78; H, 6.72.

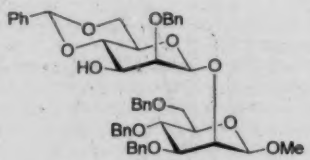


36

Methyl 3-*O*-allyl-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (36)

Compound **35** (201.2 mg, 0.267 mmol) was dissolved in dry DMF (3 mL). The mixture was cooled to 0°C (ice-water bath). Benzyl bromide (63 μL, 0.533 mmol) was added followed by sodium hydride (21.3 mg, 0.533). The reaction was quenched with methanol then concentrated under reduced pressure. Purification of the product by chromatography (4:1, hexanes-EtOAc) gave **36** (191.7 mg, 85%) as a white solid; R_f 0.56 (1:1, hexanes-EtOAc); $[\alpha]_D$ -111 (*c* 0.27, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52-7.54 (m, 2 H, ArH), 7.45-7.48 (m, 4 H, ArH), 7.37-7.37 (m, 2 H, ArH), 7.27-7.35 (m, 5 H, ArH), 7.19-7.26 (m, 10 H, ArH), 7.10-7.12 (m, 2 H, ArH), 5.85 (dddd, 1 H, *J* 5.4, 5.4, 10.5, 17.2 Hz, H-b), 5.59 (s, 1 H, PhC*H*O_2), 5.27 (dddd, 1 H, *J* 1.7, 1.7, 1.7, 17.2 Hz, H-c), 5.15 (dddd, 1 H, *J* 1.4, 1.4, 1.4, 10.5 Hz, H-d), 5.08 (d, 1 H, J_{gem} 11.8 Hz, PhCH_2O), 4.97 (d, 1 H, $J_{1',2'}$ 0.6 Hz, H-1′), 4.88 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.88 (d, 1 H, J_{gem} 11.8 Hz, PhCH_2O), 4.84 (d, 1 H, J_{gem} 10.7 Hz, PhCH_2O), 4.57 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.54 (d, 1 H, J_{gem} 10.6 Hz, PhCH_2O), 4.50 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O), 4.36 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.35 (d, 1 H, $J_{2,3}$ 2.8 Hz, H-2), 4.34 (s, 1 H, H-1), 4.29 (dd, 1 H, $J_{5',6'}$ 4.9, J_{gem} 10.4 Hz, H-6eq′), 4.18 (d, 1 H, $J_{2',3'}$ 3.9 Hz, H-2′), 4.15 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.5 Hz, H-4′), 4.09 (dddd, 1 H, *J* 1.7, 1.7, 5.3, 13.3 Hz, H-a), 4.03 (dddd, 1 H, *J* 1.5, 1.5, 5.5, 13.2 Hz, H-a′), 3.87 (dd, 1 H, $J_{5',6'} \approx J_{gem}$ 10.4 Hz, H-6ax′), 3.85 (dd, 1

H, $J_{3,4} \approx J_{4,5}$ 9.4 Hz, H-4), 3.76 (dd, 1 H, $J_{5,6}$ 1.9, J_{gem} 10.6 Hz, H-6a), 3.69 (dd, 1 H, $J_{5,6}$ 5.1 Hz, H-6b), 3.57 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.2 Hz, H-3), 3.56 (dd, 1 H, $J_{2',3'}$ 3.3, $J_{3',4'}$ 9.9 Hz, H-3'), 3.54 (s, 3 H, CH_3O), 3.44 (ddd, 1 H, $J_{4,5}$ 9.5, $J_{5,6}$ 1.8, 5.0 Hz, H-5), 3.38 (ddd, 1 H, $J_{4',5'}$ 9.4, $J_{5',6'}$ 4.9, 10.0 Hz, H-5'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.2 (Ar), 138.4(4) (Ar), 138.3(9) (Ar), 138.2 (Ar), 137.7 (Ar), 135.0 (H_2C=CH-CH_2), 128.7(4) (Ar), 128.6(7) (Ar), 128.3(2) (Ar), 128.2(6) (Ar), 128.1(8) (Ar), 128.1(3) (Ar), 128.1(1) (Ar), 127.9 (Ar), 127.8 (Ar), 127.6 (Ar), 127.5 (Ar), 127.1 (Ar), 126.1 (Ar), 116.6 (H_2C=CH-CH_2), 102.8 (C-1'), 102.2 (C-1), 101.4 (PhCHO_2), 80.5 (C-3), 78.3 (C-4'), 77.5 (C-3'), 75.7 (C-2'), 75.5 (C-5), 75.2, 74.9 (PhCH_2O×2), 74.3 (C-4), 73.4 (PhCH_2O), 73.1 (C-2), 70.8 (H_2C=CH-CH_2), 70.4 (PhCHO_2), 69.3 (C-6), 68.7 (C-6'), 67.6 (C-5'), 57.2 (CH_3O); ESI HRMS Calc'd. for $C_{51}H_{56}O_{11}Na$ 867.3715. Found 867.3710; Anal. Calc'd. for $C_{51}H_{56}O_{11}$: C, 72.49; H, 6.68. Found: C, 72.62; H, 6.76.

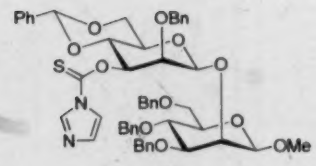


37

Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (37)

Compound **36** (900 mg, 1.07 mmol) was dissolved in dry Me_2SO (20 mL). Potassium *tert*-butoxide (640 mg, 6.39 mmol) was added then the reaction was stirred at 80°C for 20 minutes. NMR of the reaction mixture indicated complete isomerization of the allyl. The reaction was quenched with the addition of distilled H_2O then diluted with

Et_2O. The organic phase was washed with distilled H_2O, brine then finally dried (Na_2SO_4) and concentrated under reduced pressure. The residue was re-dissolved in acetone-water (9:1, 40 mL) and warmed to 40°C. Yellow mercuric oxide (347 mg, 1.60 mmol) and mercuric chloride (311 mg, 1.07 mmol) were then added. After 2 hours the reaction mixture was concentrated then subjected to chromatography (1:1, hexanes-EtOAc) to yield **37** (315 mg, 35%) as a white solid; R_f 0.31 (1:1, hexanes-EtOAc); $[\alpha]_D$ -85 (*c* 0.64, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45-7.50 (m, 6 H, ArH), 7.31-7.36 (m, 6 H, ArH), 7.24-7.27 (m, 7 H, ArH), 7.21 (m, 4 H, ArH), 7.11-7.13 (m, 2 H, ArH), 5.55 (s, 1 H, PhC*H*O_2), 5.21 (d, 1 H, J_{gem} 11.4 Hz, PhC*H*$_2$O), 5.03 (s, 1 H, H-1′), 4.90 (d, 1 H, J_{gem} 11.4 Hz, PhC*H*$_2$O), 4.83 (d, 1 H, J_{gem} 10.8 Hz, PhC*H*$_2$O), 4.74 (d, 1 H, J_{gem} 11.4 Hz, PhC*H*$_2$O), 4.58 (d, 1 H, J_{gem} 11.4 Hz, PhC*H*$_2$O), 4.54 (d, 1 H, J_{gem} 12.2 Hz, PhC*H*$_2$O), 4.47 (d, 1 H, J_{gem} 12.1 Hz, PhC*H*$_2$O), 4.33-4.35 (m, 3H, H-1, H-2, PhC*H*$_2$O), 4.31 (dd, 1 H, $J_{5',6'}$ 5.0, J_{gem} 10.4 Hz, H-6eq′), 4.16 (d, 1 H, $J_{2',3'}$ 3.9 Hz, H-2′), 3.83-3.87 (m, 3 H, H-4′, H-6ax′, H-4), 3.78 (dd, 1 H, $J_{2',3'}$ 3.7, $J_{3',4'}$ 9.8 Hz, H-3′), 3.74 (dd, 1 H, $J_{5,6}$ 1.9, J_{gem} 10.7 Hz, H-6a), 3.68 (dd, 1 H, $J_{5,6}$ 4.8, J_{gem} 10.7 Hz, H-6b), 3.59 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.3 Hz, H-3), 3.57 (s, 3 H, C*H*$_3$O), 3.44 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6}$ 1.8, 4.7 Hz, H-5), 3.38 (ddd, 1 H, $J_{4',5'}$ 9.9, $J_{5',6'}$ 5.0, 9.9 Hz, H-5′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.7 (Ar), 138.4(1) (Ar), 138.3(8) (Ar), 138.1 (Ar), 137.4 (Ar), 129.0 (Ar), 128.7 (Ar), 128.3 (Ar), 128.2(4) (Ar), 128.2(1) (Ar), 128.1 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 126.3 (Ar), 102.9 (C-1′), 102.1, 102.0 (C-1, Ph*C*HO_2), 80.6 (C-3), 79.4 (C-3′), 78.0 (C-2′), 75.6 (Ph*C*H$_2$O), 75.4 (C-5), 75.2 (Ph*C*H$_2$O), 74.3 (C-4′/C-4), 73.9 (C-2), 73.3 (Ph*C*H$_2$O), 70.6 (Ph*C*H$_2$O), 70.5 (C-4′/C-4), 69.1 (C-6′), 68.6 (C-6), 67.0 (C-5′), 57.2 (*C*H$_3$O); ESI HRMS Calc'd. for $C_{48}H_{52}O_{11}Na$ 827.3402. Found 827.3399.

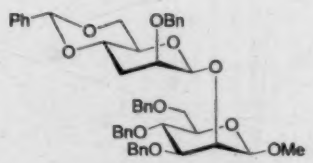


38

Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-thiocarbonylimidazole-β-D-mannopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (38)

Alcohol **37** (300 mg, 0.372 mmol) was dissolved in dry toluene (10 mL). 1,1′-Thiocarbonyl diimidazole (265 mg, 1.49 mmol) was added and the reaction was refluxed under argon fro 5 hours. The reaction mixture was cooled then concentrated under reduced pressure. The oily residue was subjected to chromatography on silica gel (3:2, hexanes-EtOAc) to give **28** (270 mg, 79%); R_f 0.37 (1:1, hexanes-EtOAc); $[\alpha]_D$ -127 (*c* 0.33, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.20 (m, 1 H, ArH), 7.51 (m, 1 H, ArH), 7.46-7.48 (m, 2 H, ArH), 7.40-7.42 (m, 2 H, ArH), 7.25-7.35 (m, 13 H, ArH), 7.10-7.18 (m, 6 H, ArH), 6.97-7.02 (m, 3 H, ArH), 6.73 (s, 1 H, PhC*H*O_2), 6.58 (dd, 1 H, $J_{2',3'}$ 3.4, $J_{3',4'}$ 10.4 Hz, H-3′), 6.04 (s, 1 H, H-1′), 5.89 (d, 1 H, J_{gem} 10.7 Hz, PhCH_2O), 5.83 (d, 1 H, J_{gem} 11.7 Hz, PhCH_2O), 5.82 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 5.70 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 5.61 (d, 1 H, $J_{2',3'}$ 3.5 Hz, H-2′), 5.58 (d, 1 H, J_{gem} 11.7 Hz, PhCH_2O), 5.31-5.36 (m, 3 H, PhCH_2O×3), 5.27 (dd, 1 H, $J_{5',6'}$ 4.4, J_{gem} 9.2 Hz, H-6eq′), 5.24 (s, 1 H, H-1), 5.10 (d, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 4.80 (dd, 1 H, $J_{5',6'} \approx J_{gem}$ 10.3 Hz, H-6b′), 4.75 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.6 Hz, H-4), 4.52 (dd, 1 H, $J_{5,6}$ 1.9, J_{gem} 1.9 Hz, H-6a), 4.46 (dd, 1 H, $J_{5,6}$ 4.7, J_{gem} 10.6 Hz, H-6b), 4.32 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.4 Hz, H-3), 4.30 (s, 3 H, CH_3O), 4.23 (ddd, 1 H, $J_{4',5'}$ 9.8, $J_{5',6'}$ 4.9, 9.8 Hz, H-5′), 4.14 (ddd, 1 H, $J_{4,5}$ 9.8, $J_{5,6}$ 1.9, 4.7 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 182.7 (C=S), 138.4 (Ar), 138.3 (Ar), 138.0(3) (Ar),

137.9(6) (Ar), 137.0 (Ar), 130.5 (Ar), 129.3 (Ar), 129.1(Ar), 128.3(2) (Ar), 128.2(7) (Ar), 128.2 (Ar), 128.1 (Ar), 127.9 (Ar), 127.7(4) (Ar), 127.6(9) (Ar), 127.6 (Ar), 127.5 (Ar), 126.1 (Ar), 103.0, 101.7, 80.5, 80.2, 75.6, 75.4(4), 75.3(7), 75.3, 74.7, 74.3, 73.4, 73.0, 70.9, 69.2, 68.6, 67.3, 56.9 (CH_3O); ESI HRMS Calc'd. for $C_{52}H_{55}N_2O_{11}S$ 915.3527. Found 915.3526.

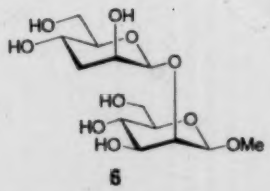


39

Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-deoxy-β-D-*arabino*-hexopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (39)

The Barton-McCombie substrate **38** (50 mg, 0.055 mmol) dissolved in dry toluene (5 mL) was added to a toluene solution (15 mL) containing tributyltin hydride (29 μL, 0.11 mmol) and azobisisobutyronitrile (2 mg, 0.11 mmol). The reaction was kept at reflux for 20 minutes The cooled reaction was filtered through a plug of silica gel containing 10% (w/w) KF then concentrated under reduced pressure. The crude product was purified by chromatography (4:1, hexanes-EtOAc) to give **39** (19 mg, 44%) as a white solid; R_f 0.53 (1:1, hexanes-EtOAc); $[\alpha]_D$ -73 (*c* 0.21, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46-7.48 (m, 5 H, ArH), 7.28-7.38 (m, 9H, ArH), 7.22-7.25 (m, 9 H, ArH), 7.12-7.13 (m, 2 H, ArH), 5.54 (s, 1 H, PhC*H*O_2), 5.07 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.99 (s, 1 H, H-1'), 4.89 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.84 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.77 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.56 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O),

4.55 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.50 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O), 4.35 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.32 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 4.32 (s, 1 H, H-1), 4.26 (dd, 1 H, $J_{5',6'}$ 4.8, J_{gem} 10.3 Hz, H-6eq'), 4.07 (ddd, 1 H, $J_{3',4'}$ 4.4, 12.0, $J_{4',5'}$ 9.2 Hz, H-4'), 4.01 (dd, 1 H, $J_{2',3'}$ 3.0 Hz, H-2'), 3.85 (dd, 1 H, $J_{5',6'} \approx J_{gem}$ 10.4 Hz, H-6ax'), 3.83 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.4 Hz, H-4), 3.75 (dd, 1 H, $J_{5,6}$ 1.8, J_{gem} 10.7 Hz, H-6a), 3.68 (dd, 1 H, $J_{5,6}$ 5.2, J_{gem} 10.7 Hz, H-6b), 3.56 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.3 Hz, H-3), 3.52 (s, 3 H, CH_3O), 3.48 (ddd, 1 H, $J_{4',5'}$ 9.9, $J_{5',6'}$ 4.9, 9.9 Hz, H-5'), 3.43 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6}$ 1.7, 5.1 Hz, H-5), 2.27 (ddd, 1 H, $J_{2',3'} \approx J_{3',4'}$ 3.8, J_{gem} 12.9 Hz, H-3eq'), 1.77 (ddd, 1 H, $J_{2',3'}$ 3.1, $J_{3,4} \approx J_{gem}$ 12.6 Hz, H-3ax'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.4 (Ar), 138.5 (Ar), 138.4 (Ar), 138.2 (Ar), 137.7 (Ar), 128.9 (Ar), 128.3 (Ar), 128.1(3) (Ar), 128.0(7) (Ar), 127.7 (Ar), 127.5(7) (Ar), 127.4(9) (Ar), 127.2 (Ar), 126.1 (Ar), 103.6 (C-1'), 102.2 (C-1), 101.8 (PhCHO$_2$), 80.6 (C-3), 75.5 (C-5), 75.1 (PhCH_2O), 74.4, 74.1 (C-4'/C-2'/C-4), 73.9(3) (PhCH_2O), 73.8(8) (C-4'/C-2'/C-4), 73.4 (C-2), 73.3 (PhCH_2O), 71.1 (C-5'), 70.4 (PhCH_2O), 69.3, 69.1 (C-6'/C-6), 57.1 (CH_3O), 34.7 (C-3'); ESI HRMS Calc'd. for $C_{48}H_{52}O_{10}Na$ 811.3453. Found 811.3437.

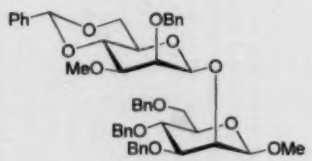


5

Methyl 3-deoxy-β-D-*arabino*-hexopyranosyl (1→2) β-D-mannopyranoside (6)

Compound **39** was dissolved in EtOAc (5 mL)-methanol (5 mL) and stirred with 10% Pd/C (50 mg) under an H_2 atmosphere then processed as described for **1**. Filtration

then lyophilization gave **6** as a clear glass; R_f 0.35 (6:3.5:0.5, CH_2Cl_2-MeOH-H_2O); $[\alpha]_D$ -90 (*c* 0.20, H_2O); ^1H NMR (500 MHz, D_2O) δ 4.80 (s, 1 H, $J_{1',2'}$ 0.9 Hz, H-1′), 4.64 (s, 1 H, H-1), 4.25 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 4.09 (dd, 1 H, $J_{2',3'}$ 2.9, 2.9 Hz, H-2′), 3.92 (dd, 1 H, $J_{5,6}$ 2.3, J_{gem} 12.4 Hz, H-6a), 3.89 (dd, 1 H, $J_{5',6'}$ 2.3, J_{gem} 12.3 Hz, H-6a′), 3.77-3.72 (m, 1 H, H-4′), 3.73 (dd, 1 H, $J_{5,6}$ 6.4, J_{gem} 12.4 Hz, H-6b), 3.70 (dd, 1 H, $J_{5',6'}$ 7.0, J_{gem} 12.4 Hz, H-6b′), 3.62 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.8 Hz, H-3), 3.57 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.6 Hz, H-4), 3.53 (s, 3 H, CH_3O-C1), 3.45 (ddd, 1 H, $J_{4',5'}$ 9.7, $J_{5',6'}$ 2.3, 6.9 Hz, H-5′), 3.39 (ddd, 1 H, $J_{4,5}$ 9.4, $J_{5,6}$ 2.3, 6.5 Hz, H-5), 2.22 (ddd, 1 H, $J_{2',3'}$ 3.7, $J_{3',4'}$ 4.9, J_{gem} 13.9 Hz, H-3′eq), 1.72 (ddd, 1 H, $J_{2',3'}$ 3.0, $J_{3',4'}$ 11.6, J_{gem} 13.8 Hz, H-3′ax); ^{13}C NMR (125 MHz, D_2O) δ 102.6 (C-1′), 102.2 (C-1), 81.0 (C-5′), 78.9 (C-2), 77.3 (C-5), 73.2 (C-3), 68.2 (C-4), 67.8 (C-2′), 62.5 (C-4′), 62.2 (C-6′), 61.8 (C-6), 58.0 (CH_3O-C1), 37.4 (C-3′); ESI HRMS Calc'd. for $C_{13}H_{24}O_{10}Na$ 363.1262. Found 363.1261.

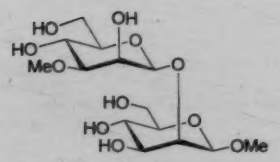


40

Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methyl-β-D-mannopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (40)

Compound **37** (46.1 mg, 0.0573 mmol) was dissolved in dry DMF (2 mL) then the mixture was cooled to 0°C (ice-water bath). Methyl iodide (7 μL, 0.11 mmol) was added followed by sodium hydride (8 mg, 0.22 mmol). The reaction was allowed to slowly warm to room temperature. After 3 hours, reaction was quenched with methanol and diluted with EtOAc. The organic phase was washed with distilled H_2O then brine,

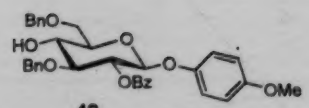
dried (Na_2SO4) and concentrated under reduced pressure. The crude mixture was purified by chromatography (7:3, hexanes-EtOAc) to give **40** (46.9 mg, quant.) as a white solid; R_f 0.44 (1:1, hexanes-EtOAc); $[\alpha]_D$ -82 (*c* 0.24, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52-7.54 (m, 2 H, ArH), 7.46-7.49 (m, 4 H, ArH), 7.20-7.37 (m, 17 H, ArH), 7.11-7.13 (m, 2 H, ArH), 5.57 (s, 1 H, PhC*H*O_2), 5.11 (d, 1 H, J_{gem} 11.8 Hz, PhCH_2O), 4.99 (s, 1 H, H-1′), 4.88 (d, 1 H, J_{gem} 11.4 Hz, PhCH_2O), 4.85 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.83 (d, 1 H, J_{gem} 10.5 Hz, PhCH_2O), 4.57 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O), 4.54 (d, 1 H, J_{gem} 11.4 Hz, PhCH_2O), 4.50 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O), 4.34-4.37 (m, 3 H, H-1, H-2, PhCH_2O), 4.29 (dd, 1 H, $J_{5',6'}$ 4.8, J_{gem} 10.3 Hz, H-6a′), 4.23 (d, 1 H, $J_{2',3'}$ 3.2 Hz, H-2′), 4.12 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.6 Hz, H-4′), 3.87 (dd, 1 H, $J_{5',6'} \approx J_{gem}$ 10.3 Hz, H-6b′), 3.84 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4), 3.75 (dd, 1 H, $J_{5,6}$ 1.9, J_{gem} 10.6 Hz, H-6a), 3.69 (dd, 1 H, $J_{5,6}$ 5.1, J_{gem} 10.6 Hz, H-6b), 3.58 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.3 Hz, H-3), 3.56 (s, 3 H, CH_3O), 3.44 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6}$ 1.8, 5.0 Hz, H-5), 3.43 (m, 2 H, H-3′, H-5′), 3.34 (s, 3 H, CH_3O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.1 (Ar), 138.4(2) (Ar), 138.3(7) (Ar), 138.2 (Ar), 137.6 (Ar), 128.9 (Ar), 128.6 (Ar), 128.2(77) (Ar), 128.2(71) (Ar), 128.2 (Ar), 128.1(6) (Ar), 128.1(5) (Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (Ar), 127.6 (Ar), 127.5(28) (Ar), 127.5(23) (Ar), 127.2 (Ar), 126.2 (Ar), 102.8 (C-1′), 102.2 (C-1), 101.7 (PhCHO_2), 80.5 (C-3), 79.8 (C-3′), 78.1 (C-4′), 75.4 (C-5), 75.2 (PhCH_2O), 74.9 (PhCH_2O), 74.6 (C-2′), 74.3 (C-4), 73.4 (PhCH_2O), 73.0 (C-2), 70.4 (PhCH_2O), 69.2 (C-6), 68.7 (C-6′), 67.6 (C-5′), 57.4 (CH_3O), 57.2 (CH_3O); ESI HRMS Calc'd. for $C_{49}H_{54}O_{11}Na$ 841.3558. Found 841.3557.



7

Methyl 3-*O*-methyl-β-D-mannopyranosyl (1→2) β-D-mannopyranoside (7)

Compound **40** (43 mg, 0.052 mmol) was dissolved in EtOAc (5 mL)-methanol (5 mL) and stirred with 10% Pd/C (45 mg) under an H_2 atmosphere then processed as described for **1**. Filtration then lyophilization gave **7** (17 mg, 89%) as a clear glass; R_f 0.34 (6:3.5:0.5, CH_2Cl_2-MeOH-H_2O); $[\alpha]_D$ -78 (*c* 0.13, H_2O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.81 (s, 1 H, H-1′), 4.65 (s, 1 H, H-1), 4.37 (d, 1 H, $J_{2',3'}$ 3.1 Hz, H-2′), 4.26 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 3.94 (dd, 1 H, $J_{5,6}$ 2.3, J_{gem} 12.3 Hz, H-6a), 3.91 (dd, 1 H, $J_{5',6'}$ 2.2, J_{gem} 12.3 Hz, H-6a′), 3.74 (dd, 1 H, $J_{5,6}$ 6.5, J_{gem} 12.7 Hz, H-6b), 3.72 (dd, 1 H, $J_{5',6'}$ 6.7, J_{gem} 12.7 Hz, H-6b′), 3.64 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.8 Hz, H-3), 3.59 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.8 Hz, H-4′), 3.58 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.7 Hz, H-4), 3.55 (s, 3 H, CH_3OC), 3.45 (s, 3 H, CH_3OC), 3.35-3.41 (m, 2 H, H-5′, H-5), 3.34 (dd, 1 H, $J_{2',3'}$ 3.2, $J_{3',4'}$ 9.6 Hz, H-3′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 102.3 (C-1), 101.4 (C-1′), 82.7 (C-3′), 78.7 (C-2), 77.3, 77.2 (C-5′/C-5), 73.2 (C-3), 68.2 (C-4′/C-4), 66.9 (C-2′), 66.6 (C-4′/C-4), 61.9(2), 61.8(5) (C-6′/C-6), 58.0 (*C*H_3O), 57.0 (*C*H_3O); ESI HRMS Calc'd. for $C_{14}H_{26}O_{11}Na$ 393.1367. Found 393.1366.

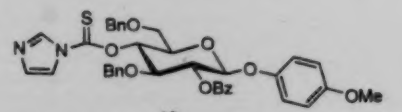


42

***p*-Methoxyphenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (42)**

To a solution of *p*-methoxyphenyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside[205] **41** (2.0 g, 3.5 mmol) and $NaCNBH_3$ (1.1 g, 17.6 mmol) in dry THF (50 mL) containing activated 4Å molecular sieves (0 °C under argon) was added dropwise a 1M solution of HCl in diethyl ether until the solution was acidic (pH paper, gas evolution). After 3 h, the ice bath was removed, the reaction diluted with EtOAc then filtered through celite. The organic phase was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO4) then concentrated under reduced pressure. The crude product was purified by column chromatography (4:1, hexanes-EtOAc) to give **42** (1.6 g, 82%) as a white solid; R_f 0.50 (1:1, hexanes-EtOAc); $[\alpha]_D$ +5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04-8.06 (m, 2 H, ArH), 7.59 (m, 1 H, ArH), 7.44-7.47 (m, 2 H, ArH), 7.30-7.37 (m, 5 H, ArH), 7.19-7.23 (m, 5 H, ArH), 6.89-6.91 (m, 2 H, ArH), 6.72-6.74 (m, 2 H, ArH), 5.49 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.6 Hz, H-2), 4.99 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.76 (d, 1 H, J_{gem} 11.7 Hz PhCH_2O), 4.70 (d, 1 H, J_{gem} 11.5 Hz PhCH_2O), 4.64 (d, 1 H, J_{gem} 11.9 Hz PhCH_2O), 4.60 (d, 1 H, J_{gem} 11.9 Hz PhCH_2O), 3.88 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.1 Hz, H-4), 3.86 (dd, 1 H, $J_{5,6}$ 4.3, J_{gem} 10.4 Hz, H-6a), 3.81 (dd, 1 H, $J_{5,6}$ 5.5, J_{gem} 10.3 Hz, H-6b), 3.75 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 9.2 Hz, H-3), 3.73 (s, 3 H, CH_3O), 3.66 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6}$ 4.3, 5.4 Hz, H-5), 2.73 (d, 1 H, $J_{4,OH}$ 1.8 Hz, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2 (*C*=O), 155.5 (Ar), 151.3 (Ar), 137.9 (Ar), 137.7 (Ar), 133.2 (Ar), 129.8 (Ar), 129.8 (Ar),

128.5 (Ar), 128.4 (Ar), 128.0 (Ar), 127.8(4) (Ar), 127.8(2) (Ar) 127.7 (Ar), 118.8 (Ar), 114.4 (Ar), 100.9 (C-1), 82.2 (C-3), 74.5 (C-5), 74.4 (Ph*C*H_2O), 73.8 (Ph*C*H_2O), 73.3 (C-2), 72.0 (C-4), 70.3 (C-6), 55.6 (*C*H_3O); ESI HRMS Calc'd. for $C_{34}H_{34}O_8Na$ 593.2146. Found 593.2143. Anal. Calc'd. for $C_{34}H_{34}O_8$: C, 71.56; H, 6.01. Found: C, 71.31; H, 5.94.

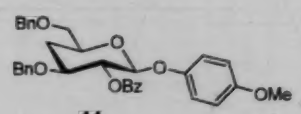


43

***p*-Methoxyphenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-thiocarbonylimidazole-β-D-glucopyranoside (43)**

To a solution of **42** (1.06 g, 1.86 mmol) in dry toluene (20 mL) was added 1,1'-thiocarbonyl diimidazole (995.0 mg, 5.58 mmol). The reaction was refluxed under argon overnight. After cooling to room temperature the reaction was concentrated under reduced pressure. The remaining yellow syrup was subjected to column chromatography (silica gel) with hexanes-EtOAc (3:2) yielding **43** (1.13 g, 89%) as a white solid; R_f 0.30 (1:1, hexanes-EtOAc); $[\alpha]_D$ -22 (*c* 1.0 $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06-8.10 (m, 3 H, ArH), 7.61 (m, 1 H, ArH), 7.46-7.49 (m, 3 H, ArH), 7.24-7.27 (m, 5 H, ArH), 6.93-7.20 (m, 8 H, ArH), 6.76-6.73 (m, 2 H, ArH), 6.07 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.2 Hz, H-4), 5.65 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 8.9 Hz, H-2), 5.13 (d, 1 H, H-1), 4.64 (d, 1 H, J_{gem} 11.8 Hz, PhCH_2O), 4.50 (d, 1 H, J_{gem} 11.6 Hz, PhCH_2O), 4.46 (m, 2 H, PhCH_2O), 4.12 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 8.9 Hz, H-3), 3.96 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6}$ 4.0, 5.8 Hz, H-5), 3.75 (dd, 1 H, $J_{5,6}$ 4.1 Hz, H-6a), 3.74 (s, 3 H, CH_3O), 3.69 (dd, 1 H, $J_{5,6}$ 5.8, J_{gem} 10.4 Hz, H-6b); ^{13}C NMR

(125 MHz, $CDCl_3$) δ 182.9 (*C*=S), 164.9 (*C*=O), 155.7 (Ar), 151.1 (Ar), 137.4 (Ar), 136.7 (Ar), 136.6 (Ar), 133.5 (Ar), 130.9 (Ar), 129.8 (Ar), 129.4 (Ar), 128.6 (Ar), 128.3 (Ar), 128.2(4) (Ar), 128.2(1) (Ar), 128.1(4) (Ar), 128.0(7) (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.6 (Ar), 118.7 (Ar), 118.1 (Ar), 114.5 (Ar), 100.5 (C-1), 78.9, 78.8 (C-3, C-4), 74.1 (Ph*C*H_2O), 73.9 (Ph*C*H_2O), 73.5, 73.3 (C-2, C-5), 69.7 (C-6), 55.6 (*C*H_3O); ESI HRMS Calc'd. for $C_{38}H_{36}N_2O_8SNa$ 703.2085. Found 703.2090; Anal. Calc'd. for $C_{38}H_{36}N_2O_8S$: C, 67.04; H, 5.33; N, 4.11; S, 4.71. Found: C, 67.28; H, 5.44; N, 4.11; S, 4.67.

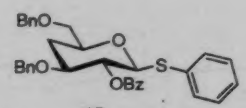


44

***p*-Methoxyphenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-deoxy-β-D-*xylo*-hexopyranoside (44)**

The Barton-McCombie substrate **43** (1.0 g, 1.47 mmol) was dissolved in dry toluene (20 mL). Tributyltin hydride (1.2 mL, 4.41 mmol) and azobisisobutyronitrile (60 mg, 0.37 mmol) were added and the reaction was refluxed under argon overnight. The cooled reaction was filtered through a plug of silica gel containing 10% (w/w) KF then concentrated under reduced pressure. The crude product was purified by chromatography (4:1, hexanes-EtOAc) to give **44** (652.7 mg, 80%) a white solid; R_f 0.60 (1:1, hexanes-EtOAc); $[\alpha]_D$ +14 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06-8.08 (m, 2 H, ArH), 7.59 (m, 1 H, ArH), 7.44-7.48 (m, 2 H, ArH), 7.30-7.39 (m, 5 H, ArH), 7.18-7.22 (m, 5 H, ArH), 6.92-6.95 (m, 2 H, ArH), 6.72-6.75 (m, 2 H, ArH), 5.43 (dd, 1 H, $J_{1,2}$ 7.9,

$J_{2,3}$ 9.2 Hz, H-2), 4.95 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.67 (d, 1 H, J_{gem} 12.3 Hz, PhCH_2O), 4.63 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.58 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.53 (d, 1 H, J_{gem} 12.3 Hz, PhCH_2O), 3.79-3.84 (m, 2 H, H-3, H-5), 3.73 (s, 3 H, CH_3O), 3.71 (dd, 1 H, $J_{5,6}$ 6.0, J_{gem} 10.1 Hz, H-6a), 3.62 (dd, 1 H, $J_{5,6}$ 4.8, J_{gem} 10.1 Hz, H-6b), 2.29 (ddd, 1 H, J_{gem} 13.0, J 1.8, 5.1 Hz, H-4eq), 1.72 (ddd, 1 H, $J_{3,4} \approx J_{4,5}$ 11.6 Hz, H-4ax); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.5 (*C*=O), 155.3 (Ar), 151.6 (Ar), 138.1 (Ar), 137.9 (Ar), 133.0 (Ar), 130.1 (Ar), 129.8 (Ar), 128.4(3) (Ar), 128.3(6) (Ar), 128.3(0) (Ar), 127.7(4) (Ar), 127.7(2) (Ar), 127.6(1) (Ar), 127.6(0) (Ar), 118.7 (Ar), 114.4 (Ar), 101.1 (C-1), 75.9 (C-3/C-5), 74.5 (C-2), 73.6 (Ph*C*H_2O), 72.3 (C-6), 71.7 (C-3/C-5), 71.1 (Ph*C*H_2O), 55.6 (*C*H_3O), 33.1 (C-4); ESI HRMS Calc'd. for $C_{34}H_{34}O_7Na$ 577.2197. Found 577.2193; Anal. Calc'd. for $C_{34}H_{34}O_7$: C, 73.63; H, 6.18. Found: C, 73.30; H, 6.30.

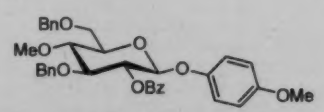


45

Phenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-deoxy-1-thio-β-D-*xylo*-hexopyranoside (45)

Compound **44** (494.3 mg, 0.89 mmol) was mixed with thiophenol (183 μL, 1.78 mmol) in dry CH_2Cl_2 (5 mL). $BF_3{\cdot}OEt_2$ (106 μL, 0.84 mmol) was added dropwise and the reaction was stirred at 0 °C under argon. After 3 h, the reaction was quenched with Et_3N and diluted with CH_2Cl_2. The organic phase was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4) then concentrated under reduced pressure. Purification of the crude product by chromatography (95:5, toluene-EtOAc) followed by recrystallization (EtOAc-hexanes) yielded **45** (442.6 mg, 92%) as a white crystalline solid; R_f 0.61 (1:1,

hexanes-EtOAc); $[\alpha]_D$ +24 (*c* 0.34, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 8.06-8.08 (m, 2 H, ArH), 7.59-7.62 (m, 1 H, ArH), 7.46-7.49 (m, 4 H, ArH), 7.30-7.38 (m, 5 H, ArH), 7.15-7.24 (m, 8 H, ArH), 5.21 (dd, 1 H, $J_{1,2}$ 9.9, $J_{2,3}$ 9.0 Hz, H-2), 4.78 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 4.62 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O), 4.60 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.57 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.49 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O), 3.73-3.78 (m, 2 H, H-3, H-5), 3.70 (dd, 1 H, $J_{5,6}$ 6.1, J_{gem} 10.1 Hz, H-6a), 3.57 (dd, 1 H, $J_{5,6}$ 4.5, J_{gem} 10.1 Hz, H-6b), 2.27 (ddd, 1 H, J_{gem} 13.0, *J* 1.9, 5.1 Hz, H-4eq), 1.65 (ddd, 1 H, $J_{3,4} \approx J_{4,5}$ 11.5, J_{gem} 13.0 Hz, H-4ax); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.4 (*C*=O), 138.1 (Ar), 137.9 (Ar), 133.4 (Ar), 133.1 (Ar), 132.3 (Ar), 130.1 (Ar), 129.9 (Ar), 128.8 (Ar), 128.4(3) (Ar), 128.3(6) (Ar), 127.7 (Ar), 127.6 (Ar), 86.6 (C-1), 77.2 (C-3), 75.5 (C-5), 73.6 (Ph*C*H_2O), 73.1 (C-2), 72.4 (C-6), 71.1 (Ph*C*H_2O), 33.4 (C-4); ESI HRMS Calc'd. for $C_{33}H_{32}O_5SNa$ 563.1863. Found 563.1867; Anal. Calc'd. for $C_{35}H_{36}O_8$: C, 73.31; H, 5.97. Found: C, 73.43; H, 6.00.

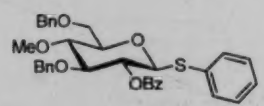


46

***p*-Methoxyphenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-methyl-β-D-glucopyranoside (46)**

Substrate **42** (1.0 g, 1.75 mmol) was dissolved in DMF and the mixture was cooled to 0 °C. Methyl iodide (218 μL, 3.51 mmol) followed by sodium hydride (140 mg, 3.51 mmol) were added and the mixture was stirred for 2 h. The reaction was quenched with acetic acid then concentrated under reduced pressure. Purification of the

product by chromatography (7:3, hexanes-EtOAc) gave **46** (898.6 mg, 88%) as a white solid; R_f 0.59 (1:1, hexanes-EtOAc); $[\alpha]_D$ +37 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.01-8.03 (m, 2 H, ArH), 7.57 (m, 1 H, ArH), 7.42-7.46 (m, 5 H, ArH), 7.26-7.38 (m, 5 H, ArH), 7.15-7.20 (m, 5 H, ArH), 6.91-6.94 (m, 2 H, ArH), 6.72-6.74 (m, 2 H, ArH), 5.47 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 9.5 Hz, H-2), 4.95 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.78 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.68 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.67 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.61 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 3.86 (dd, 1 H, $J_{5,6}$ 1.8, J_{gem} 10.9 Hz, H-6a), 3.76-3.80 (m, 2 H, H-3, H-6b), 3.73 (s, 3 H, CH_3O), 3.60 (ddd, 1 H, $J_{4,5}$ 9.9, $J_{5,6}$ 1.8, 5.1 Hz, H-5), 3.56 (s, 3 H, CH_3O), 3.54 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2 (*C*=O), 155.4 (Ar), 151.5 (Ar), 138.2 (Ar), 137.8 (Ar), 133.1 (Ar), 129.9 (Ar), 129.8 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 127.6(6) (Ar), 127.6(4) (Ar), 127.5(8) (Ar), 118.8 (Ar), 114.4 (Ar), 100.8 (C-1), 82.5 (C-3), 80.0 (C-4), 75.6 (C-5), 74.9 (Ph*C*H_2O), 73.6, 73.5 (C-2, PhCH_2O), 68.9 (C-6), 60.8 (*C*H_3O), 55.6 (*C*H_3O); ESI HRMS Calc'd. for $C_{35}H_{36}O_8Na$ 607.2302. Found 607.2304. Anal. Calc'd. for $C_{35}H_{36}O_8$: C, 71.90; H, 6.21. Found: C, 71.95; H, 6.22.

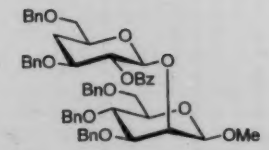


47

Phenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-methyl-1-thio-β-D-glucopyranoside (47)

Compound **46** (529.8 mg, 0.91 mmol) was mixed with thiophenol (186 μL, 1.81 mmol) in dry CH_2Cl_2 (5 mL). $BF_3{\cdot}OEt_2$ (138 μL, 1.09 mmol) was added dropwise and the reaction was stirred at 0 °C (ice-water bath) under argon. After 3 h, the reaction was

quenched with Et_3N and diluted with CH_2Cl_2. The organic phase was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4) then concentrated under reduced pressure. Purification of the crude product by chromatography (95:5, toluene-EtOAc) followed by recrystallization (EtOAc-hexanes) yielded **47** (350.9 mg, 68%) as a white crystalline solid; R_f 0.68 (1:1, hexanes-EtOAc); $[\alpha]_D$ +44 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03-8.05 (m, 2 H, ArH), 7.59 (m, 1 H, ArH), 7.44-7.50 (m, 4 H, ArH), 7.30-7.38 (m, 5 H, ArH), 7.19-7.25 (m, 3 H, ArH), 7.13-7.16 (m, 5 H, ArH), 5.24 (dd, 1 H, $J_{1,2}$ 10.0, $J_{2,3}$ 9.2 Hz, H-2), 4.77 (d, 1 H, $J_{1,2}$ 10.1 Hz, H-1), 4.74 (d, 1 H, J_{gem} 11.2 Hz, PhCH_2O), 4.66 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.64 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.60 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 3.85 (dd, 1 H, $J_{5,6}$ 1.9, J_{gem} 11.0 Hz, H-6a), 3.78 (dd, 1 H, $J_{5,6}$ 4.9, J_{gem} 11.0 Hz, H-6b), 3.74 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 9.0 Hz, H-3), 3.54 (ddd, 1 H, $J_{4,5}$ 9.8, $J_{5,6}$ 1.9, 5.0 Hz, H-5), 3.53 (s, 3 H, CH_3O), 3.45 (dd, 1 H, $J_{3,4}$ 8.9, $J_{4,5}$ 9.7 Hz, H-4); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.1 (*C*=O), 138.3 (Ar), 137.8 (Ar), 133.1 (Ar), 133.0 (Ar), 132.5 (Ar), 130.0 (Ar), 129.9 (Ar), 128.8 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.0 (Ar), 127.7 (Ar), 127.6(2) (Ar), 127.5(6) (Ar), 86.1 (C-1), 84.0 (C-3), 79.9 (C-4), 79.6 (C-5), 75.1 (PhCH_2O), 73.5 (PhCH_2O), 72.3 (C-2), 69.1 (C-6), 60.8 (CH_3O); ESI HRMS Calc'd. for $C_{34}H_{34}O_6SNa$ 593.1968. Found 593.1961. Anal. Calc'd. for $C_{34}H_{34}O_6S$: C, 71.56; H, 6.00; S, 5.62. Found: C, 71.79; H, 6.08; S, 5.47.

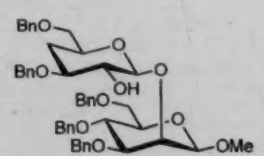


48

Methyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-deoxy-β-D-*xylo*-hexopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (48)

Glycosyl donor **45** (250.0 mg, 0.46 mmol) and methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside[183] **20** (236.3 mg, 0.51 mmol) were dried together under vacuum for 2 h in a pear shaped flask (25 mL). The contents of the flask were then dissolved in dry CH_2Cl_2 (5 mL), activated 4Å molecular sieves were added and the solution was cooled to 0 °C (ice-water bath) under argon. N-Iodosuccinimide (124.8 mg, 0.55 mmol) was added followed by silver triflate (24 mg, 0.09 mmol). After 2 h, the reaction was neutralized with Et_3N and filtered through celite. The mixture was diluted with CH_2Cl_2 and washed with 10% aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$, distilled water then brine. The organic phase was dried (Na_2SO_4) then concentrated under reduced pressure. Purification of the product by chromatography (95:5, toluene-EtOAc) gave **48** (310.0 mg, 75%) as a clear syrup: R_f 0.61 (1:1, hexanes-EtOAc); $[\alpha]_D$ -49 (*c* 0.52, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07-8.09 (m, 2 H, ArH), 7.13-7.48 (m, 28 H, ArH), 5.30 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.3 Hz, H-2′), 4.91-4.93 (m, 2 H, H-1′, PhCH_2O), 4.87 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.65 (d, 1 H, J_{gem} 12.5 Hz, PhCH_2O), 4.46-4.56 (m, 5 H, 5(PhCH_2O)), 4.23 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 4.18 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.11 (s, 1 H, H-1), 4.05 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 3.79 (ddd, 1 H, $J_{2',3'}$ 9.3, $J_{3',4ax'}$ 11.3, $J_{3',4eq'}$ 5.1 Hz, H-3′), 3.65-3.76

(m, 3 H, H-5′, H-6a′, H-6a), 3.59 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.2 Hz, H-4), 3.52 (dd, 1 H, $J_{5',6'}$ 4.5, J_{gem} 9.3 Hz, H-6b′), 3.43 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.3 Hz, H-3), 3.29 (ddd, 1 H, $J_{5,6}$ 1.3, 7.1 Hz, H-5), 3.26 (3, s, CH_3O), 3.24 (dd, 1 H, $J_{5,6}$ 7.1, J_{gem} 10.7 Hz, H-6b), 2.21 (ddd, 1 H, $J_{3',4'}$ 4.9, J_{gem} 12.6, $J_{4',5'}$ 1.4 Hz, H-4eq′), 1.65 (ddd, 1 H, $J_{4',5'}$ 12.6 Hz, H-4ax′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.5 (*C*=O), 138.7 (Ar), 138.6 (Ar), 138.4 (Ar), 138.3 (Ar), 137.9 (Ar), 132.3 (Ar), 131.1 (Ar), 130.1 (Ar), 128.4 (Ar), 128.2(4) (Ar), 128.2(3) (Ar), 128.1(2) (Ar), 128.1(0) (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.6 (Ar), 127.4(9) (Ar), 127.4(7) (Ar), 127.4(2) (Ar), 127.2 (Ar), 101.6, 101.4 (C-1′, C-1), 80.2 (C-3), 76.2 (C-3′), 76.0 (C-5′), 75.2 (Ph*C*H_2O), 74.9, 74.8 (C-2, C-4), 73.6 (Ph*C*H_2O), 73.3, 72.8 (C-6, Ph*C*H_2O), 72.3 (C-2), 71.0, 70.9 (C-5, Ph*C*H_2O), 70.5, 70.1 (C-6′, Ph*C*H_2O), 56.6 (*C*H_3O), 33.7 (C-4′); ESI HRMS Calc'd. for $C_{55}H_{58}O_{11}Na$ 917.3871. Found 917.3878; Anal. Calc'd. for $C_{55}H_{58}O_{11}$: C, 73.81; H, 6.53. Found: C, 73.46; H, 6.51.

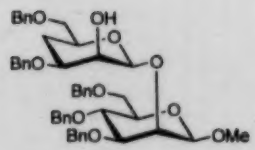


49

Methyl 3,6-di-*O*-benzyl-4-deoxy-β-D-*xylo*-hexopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (49)

Disaccharide **48** (295 mg, 0.33 mmol) was dissolved in a mixture of CH_2Cl_2 (5mL) and methanol (5 mL) then treated with 0.5 M MeONa/MeOH (2 mL) then processed as described for **23**. Purification of the product by chromatography over silica gel (1:1, hexanes-EtOAc) gave **49** (200 mg, 77%) as a clear syrup; R_f 0.23 (1:1, hexanes-

EtOAc); $[\alpha]_D$ -46 (*c* 0.60, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.24-7.40 (m, 23 H, ArH), 7.18-7.20 (m, 2 H, ArH), 4.93 (d, 1 H, J_{gem} 10.7 Hz, PhCH_2O), 4.87 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.84 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.72 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.63 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.57 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.57 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.56 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1′), 4.51 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.50 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.46 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.29 (s, 1 H, H-1), 4.21 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 3.88 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4), 3.78 (dd, 1 H, $J_{5,6}$ 2.1, J_{gem} 11.0 Hz, H-6a), 3.74 (dd, 1 H, $J_{5,6}$ 5.2, J_{gem} 11.0 Hz, H-6b), 3.67 (dddd, 1 H, $J_{4ax',5'}$ 11.4, $J_{4eq',5'}$ 1.8, $J_{5',6'}$ 4.6, 6.4 Hz, H-5′), 3.57-3.65 (m, 3 H, H-2′, H-3′, H-6a′), 3.51-3.53 (m, 4 H, H-3, CH_3O), 3.46 (dd, 1 H, $J_{5,6}$ 4.7, J_{gem} 9.7 Hz, H-6b′), 3.41 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6}$ 2.0, 5.2 Hz, H-5), 2.06 (ddd, 1 H, $J_{3',4'}$ 5.1, J_{gem} 13.1, $J_{4',5'}$ 1.8 Hz, H-4_{eq}′), 1.51 (ddd, 1 H, $J_{3',4'}$ 11.5, J_{gem} 12.8, $J_{4',5'}$ 11.4 Hz, H-4ax′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.1 (Ar), 138.5 (Ar), 138.3 (Ar), 138.1 (Ar), 138.0 (Ar), 128.4 (Ar), 128.3(4) (Ar), 128.3(0) (Ar), 128.2(8) (Ar), 128.0 (Ar), 127.7(3) (Ar), 127.6(8) (Ar), 127.6(1) (Ar), 127.5(7) (Ar), 127.5(4) (Ar), 127.3 (Ar), 104.9 (C-1′), 101.5 (C-1), 80.3 (C-3), 78.2 (C-3′), 75.8 (C-2′, C5), 75.2, 74.9, 74.6 (C-2, C-4, PhCH_2O), 73.5, 73.4, 72.7 (C-6′, PhCH_2O×2), 72.0 (PhCH_2O), 71.4 (C-5′), 70.5, 69.2 (C-6, PhCH_2O), 57.0 (CH_3O), 33.5 (C-4′); ESI HRMS Calc'd. for $C_{48}H_{54}O_{10}Na$ 813.3609. Found 813.3607.

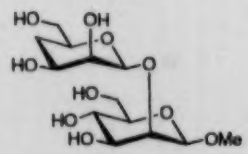


50

Methyl 3,6-di-*O*-benzyl-4-deoxy-β-D-*lyxo*-hexopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (50)

Methyl disaccharide **49** (185.5 mg, 0.24 mmol) was dissolved in a mixture of Me_2SO (5 mL) and acetic anhydride (2.5 mL) then processed as described for **24**. The oxidation product was redissolved in dry THF, cooled to -78 °C under an atmosphere of argon and reacted with L-Selectride® (1 M) in THF (1.2 mL, 1.18 mmol). Purification of the product by chromatography (7:3, hexanes-EtOAc) gave **50** (97.5 mg, 73%) as a clear syrup; R_f 0.39 (1:1, hexanes-EtOAc); $[\alpha]_D$ -67 (*c* 0.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25-7.39 (m, 23 H, ArH), 7.14-7.16 (m, 2 H, ArH), 4.90 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.87 (d, 1 H, J_{gem} 10.7 Hz, PhCH_2O), 4.77 (d, 1 H, $J_{1',2'}$ 0.9 Hz, H-1′), 4.72 (d, 1 H, J_{gem} 12.5 Hz, PhCH_2O), 4.63 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.61 (d, 1 H, J_{gem} 12.4 Hz, PhCH_2O), 4.56 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.43-4.50 (m, 5 H, H-2, PhCH_2O×4), 4.28 (d, 1 H, $J_{1,2}$ 0.6 Hz, H-1), 4.25 (d, 1 H, $J_{2',3'}$ 2.5 Hz, H-2′), 3.78-3.82 (m, 2 H, H-4, H-6a), 3.72 (dd, 1 H, $J_{5,6}$ 5.6, J_{gem} 10.8 Hz, H-6b), 3.58-3.65 (m, 2 H, H-5′, H-6a′), 3.53 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 9.3 Hz, H-3), 3.46-3.52 (m, 5 H, H-3′, H-6b′, CH_3O), 3.43 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6}$ 1.9, 5.5 Hz, H-5), 2.74 (bs, 1 H, OH), 1.86 (ddd, 1 H, $J_{3',4'} \approx J_{4',5'} \approx J_{gem}$ 11.9 Hz, H-4ax′), 1.76 (ddd, 1 H, *J* 2.1, 4.8, J_{gem} 12.2 Hz, H-4eq′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4 (Ar), 138.2(9) (Ar), 138.2(8) (Ar), 138.2 (Ar), 138.0 (Ar), 128.4 (Ar),

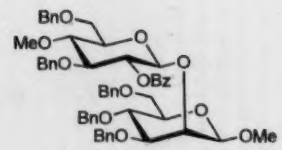
128.3(4) (Ar), 128.2(6) (Ar), 128.1(4) (Ar), 128.0(6) (Ar), 127.9 (Ar), 127.6(9) (Ar), 127.6(7) (Ar), 127.6 (Ar), 127.5 (Ar), 102.1 (C-1), 99.7 (C-1′), 80.4 (C-3), 75.6 (C-5), 75.1, 74.9 (C-3′, PhCH$_2$O), 74.1 (C-4), 73.4(9) (PhCH$_2$O), 73.4(6) (PhCH$_2$O), 73.1 (C-6′), 71.5, 70.3 (C-5′, C-2), 70.1, 69.4 (C-6, PhCH$_2$O×2), 66.7 (C-2′), 57.2 (CH$_3$O), 28.4 (C-4′); ESI HRMS Calc'd. for $C_{48}H_{54}O_{10}Na$ 813.3609. Found 813.3622.



8

Methyl 4-deoxy-β-D-*lyxo*-hexopyranosyl (1→2) β-D-mannopyranoside (8)

As described for **1**, compound **8** was prepared from **50** (49.8 mg, 0.063 mmol) in CH_2Cl_2 (5 mL)-methanol (5 mL) and 10% Pd/C (50 mg) under a H_2 atmosphere, to give **8** (15 mg, 72%) as a white amorphous solid; R_f 0.29 (6:3.5:0.5, CH_2Cl_2-MeOH-H_2O); $[\alpha]_D$ -67 (*c* 0.51, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 4.64-4.65 (m, 2 H, H-1′, H-1), 4.24 (d, 1 H, $J_{2,3}$ 2.8 Hz, H-2), 4.01 (d, 1 H, $J_{2',3'}$ 2.9 Hz, H-2′), 3.94 (dd, 1 H, $J_{5,6}$ 2.2, J_{gem} 12.3 Hz, H-6a), 3.89 (ddd, 1 H, $J_{2',3'}$ 3.0, $J_{3',4eq'}$ 5.1, $J_{3',4ax'}$ 11.9 Hz, H-3′), 3.75 (dd, 1 H, $J_{5,6}$ 6.5, J_{gem} 12.4 Hz, H-6b), 3.57-3.70 (m, 5 H, H-5′, H-6a′, H-6b′, H-3, H-4), 3.55 (s, 3 H, C*H*$_3$O-C1), 3.40 (ddd, 1 H, $J_{4,5}$ 9.1, $J_{5,6}$ 2.3, 6.6 Hz, H-5), 1.67 (ddd, 1 H, $J_{3',4'}$ 5.0, $J_{4',5'}$ 1.8, J_{gem} 12.1 Hz, H-4ax′), 1.51 (ddd, 1 H, $J_{3',4'} \approx J_{4',5'} \approx J_{gem}$ 12.0 Hz, H-4eq′); ^{13}C NMR (125 MHz, D_2O) δ 102.1, 101.9 (C-1′, C-1), 78.9 (C-2), 77.3 (C-5), 73.8, 73.2 (C-3/C-4/C-5′), 69.8 (C-2′), 68.6 (C-3′), 68.3 (C-3/C-4/C-5′), 64.7 (C-6′), 61.8 (C-6), 58.0 (CH$_3$O), 29.4 (C-4′); ESI HRMS Calc'd. for $C_{13}H_{24}O_{10}Na$ 363.1262. Found 363.1266.

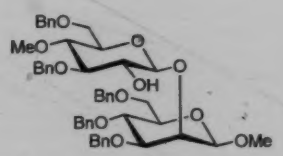


51

Methyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-methyl-β-D-glucopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (51)

Compound **47** (250 mg, 0.44 mmol) and methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside[183] **20** (224 mg, 0.48 mmol) in dry CH_2Cl_2 (5 mL) were reacted with N-Iodosuccinimide (118 mg, 0.53 mmol) and silver triflate (23 mg, 0.09 mmol) then processed as described for **48**. The product was purified by column chromatography over silica gel (95:5, toluene-EtOAc) to give **51** (344 mg, 85%) as a clear syrup; R_f 0.63 (1:1, hexanes-EtOAc); $[\alpha]_D$ -39 (*c* 0.14, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04-8.06 (m, 2 H, ArH), 7.45 (m, 1 H, ArH), 7.12-7.37 (m, 27 H, ArH), 5.35 (dd, 1 H, $J_{1',2'}$ 8.1, $J_{2',3'}$ 9.5 Hz, H-2′), 4.98 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1′), 4.91 (m, 2 H, PhCH_2O), 4.75 (d, 1 H, J_{gem} 11.4 Hz, PhCH_2O), 4.70 (d, 1 H, J_{gem} 11.4 Hz, PhCH_2O), 4.56 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.52 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.50 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.46 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.26 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 4.17 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.12 (s, 1 H, H-1), 4.03 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 3.74-3.82 (m, 3 H, H-3′, H-6a′, H-6b′), 3.64 (dd, 1 H, $J_{5,6}$ 1.4, J_{gem} 10.9 Hz, H-6a), 3.52-3.59 (m, 2 H, H-4, H-5′), 3.53 (s, 3 H, CH_3O), 3.43 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.2 Hz, H-3), 3.39 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.3 Hz, H-4′), 3.27-3.30 (m, 4 H, H-5, CH_3O), 3.21 (dd, 1 H, $J_{5,6}$ 7.1, J_{gem} 11.1 Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.3 (*C*=O), 138.7 (Ar), 138.6 (Ar), 138.2(2) (Ar), 138.2(1) (Ar), 138.1 (Ar), 132.4 (Ar), 130.9 (Ar), 130.1 (Ar), 128.3 (Ar),

128.2(4) (Ar), 128.2(3) (Ar), 128.1(6) (Ar), 128.1(2) (Ar), 128.0 (Ar), 127.9(3) (Ar), 127.8(8) (Ar), 127.7 (Ar), 127.6 (Ar), 127.4(7) (Ar), 127.4(5) (Ar), 127.2 (Ar), 101.6 (C-1), 101.1 (C-1′), 82.8 (C-3′), 80.5, 80.0 (C-4′, C-3), 76.2 (C-5), 75.2, 75.0 (C-5′, C-4), 74.8 (PhCH$_2$O), 74.6 (PhCH$_2$O), 73.8, 73.6, 73.3 (C-2′, PhCH$_2$O×2), 72.2 (C-2), 70.4 (C-6), 70.1, 69.8 (C-6′, PhCH$_2$O), 60.8 (CH$_3$O), 56.6 (CH$_3$O); ESI HRMS Calc'd. for $C_{56}H_{60}O_{12}Na$ 947.3977. Found 947.3971; Anal. Calc'd. for $C_{56}H_{60}O_{12}$: C, 72.71; H, 6.54. Found: C, 73.06; H, 6.71.

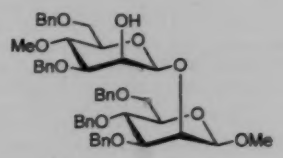


52

Methyl 3,6-di-*O*-benzyl-4-*O*-methyl-β-D-glucopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (52)

Disaccharide **51** (217 mg, 0.24 mmol) was dissolved in CH_2Cl_2-methanol (1:1, 4 mL) and treated with 0.5 M MeONa/MeOH (1 mL) then processed as described for **23**. Purification of the product by chromatography over silica gel (7:3, hexanes-EtOAc) gave **52** (181 mg, 94%) as a clear syrup; R_f 0.38 (1:1, hexanes-EtOAc); $[\alpha]_D$ +9 (*c* 0.59, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42-7.46 (m, 2 H, ArH), 7.22-7.38 (m, 21 H, ArH), 7.18-7.19 (m, 2 H, ArH), 5.03 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.93 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.89 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.79 (d, 1 H, J_{gem} 11.4 Hz, PhCH_2O), 4.61 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.64-4.47 (m, 6 H, PhCH_2O×6), 4.29 (s, 1 H, H-1), 4.23 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 3.88 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.4 Hz, H-4), 3.67-3.79 (m,

5 H, H-2′, H-6a′, H-6b′, H-6a, H-6b), 3.56 (dd, 1 H, $J_{2',3'} \approx J_{3',4'}$ 8.8 Hz, H-3′), 3.53 (s, 3 H, CH_3O), 3.53 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.2 Hz, H-3), 3.50 (s, 3 H, CH_3O), 3.48 (ddd, 1 H, $J_{4',5'}$ 9.8, $J_{5',6'}$ 2.7, 5.3 Hz, H-5′), 3.41 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6}$ 2.0, 3.4 Hz, H-5), 3.32 (bs, 1 H, OH), 3.24 (dd, 1 H, $J_{3',4'}$ 8.8, $J_{4',5'}$ 9.9 Hz, H-4′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.2 (Ar), 138.4 (Ar), 138.3 (Ar), 138.2 (Ar), 138.0 (Ar), 128.3(3) (Ar), 128.3(0) (Ar), 128.2(7) (Ar), 128.2 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.6(9) (Ar), 127.6(8) (Ar), 127.6(0) (Ar), 127.5(7) (Ar), 127.5(3) (Ar), 127.4(9) (Ar), 127.4 (Ar), 104.2 (C-1′), 101.5 (C-1), 85.0 (C-3′), 80.2 (C-3), 79.4 (C-4′), 75.7 (C-5), 75.4, 75.2(3), 75.2(1) (C-2′, C-5′, Ph*C*H_2O), 74.7, 74.6, 74.4 (C-2, C-4, Ph*C*H_2O), 73.5 (Ph*C*H_2O), 73.4 (Ph*C*H_2O), 70.4 (Ph*C*H_2O), 69.9, 69.3 (C-6′, C-6), 60.9 (*C*H_3O), 57.1 (*C*H_3O); ESI HRMS Calc'd. for $C_{49}H_{56}O_{11}Na$ 843.3715. Found 843.3718; Anal. Calc'd. for $C_{49}H_{56}O_{11}$: C, 71.69; H, 6.88. Found: C, 71.60; H, 6.90.

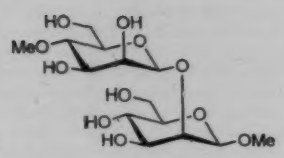


53

Methyl 3,6-di-*O*-benzyl-4-*O*-methyl-β-D-mannopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (53)

Same procedure as described for compound **24**. Disaccharide **52** (168 mg, 0.20 mmol) was stirred in a mixture of Me_2SO (5 mL) and acetic anhydride (2.5 mL). Following concentration under reduced pressure the residue was reacted with L-Selectride® (2 ml, 1.0 M solution in THF) in dry THF (5 mL) at -78 °C to give **53** (123

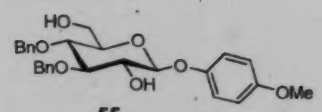
mg, 73%) as a colourless syrup; R_f 0.36 (1:1, hexanes-EtOAc); $[\alpha]_D$ -65 (*c* 0.49, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23-7.43 (m, 23 H, ArH), 7.13-7.15 (m, 2 H, ArH), 4.93 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.89 (d, 1 H, $J_{1',2'}$ 0.9 Hz, H-1′), 4.86 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.82 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.65 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O), 4.63 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.55 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.51 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.48 (d, 1H, $J_{2,3}$ 3.3 Hz, H-2), 4.46 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.44-4.46 (m, 2 H, PhCH_2O), 4.29 (d, 1 H, $J_{2',3'}$ 3.0 Hz, H-2′), 4.28 (s, 1 H, H-1), 3.76-3.80 (m, 3 H, H-6a′, H-4, H-6a), 3.71 (dd, 1 H, $J_{5,6}$ 5.4, J_{gem} 10.7 Hz, H-6b), 3.68 (dd, 1 H, $J_{5',6'}$ 6.1, J_{gem} 10.5 Hz, H-6b′), 3.61 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.3 Hz, H-4′), 3.56 (s, 3 H, CH_3O), 3.54 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 9.3 Hz, H-3), 3.50 (s, 3 H, CH_3O), 3.46 (dd, 1 H, $J_{2',3'}$ 3.0, $J_{3',4'}$ 9.1 Hz, H-3′), 3.40-3.46 (m, 2 H, H-5′, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.3(5) (Ar), 138.2(7) (Ar), 138.2(5) (Ar), 138.2 (Ar), 138.1 (Ar), 128.3(4) (Ar), 128.2(9) (Ar), 128.2(6) (Ar), 128.2 (Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.5(8) (Ar), 127.5(6) (Ar), 127.5 (Ar), 102.1 (C-1), 99.0 (C-1′), 81.2 (C-3′), 80.3 (C-2), 76.3 (C-4′), 75.6, 75.1 (C-5′, C-5, Ph*C*H_2O), 74.0 (C-4), 73.5 (Ph*C*H_2O), 73.4 (Ph*C*H_2O), 70.7 (Ph*C*H_2O), 70.3, 70.2, 70.0, 69.4 (C-6′, C-2, C-6, Ph*C*H_2O), 67.8 (C-2′), 60.9 (*C*H_3OC-4′), 57.2 (*C*H_3OC-1); ESI HRMS Calc'd. for $C_{49}H_{56}O_{11}Na$ 843.3715. Found 843.3719; Anal. Calc'd. for $C_{49}H_{56}O_{11}$: C, 71.69; H, 6.88. Found: C, 71.31; H, 6.64.



9

Methyl 4-*O*-methyl-β-D-mannopyranosyl (1→2) β-D-mannopyranoside (9)

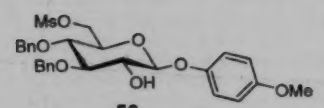
Compound **53** (43.0 mg, 0.052 mmol) was dissolved in CH_2Cl_2 (5 mL)-methanol (5 mL) and stirred with 10% Pd/C (50 mg) under an H_2 atmosphere then processed as described for **1**. Filtration then lyophilization gave **9** (12.1 mg, 62%) as a white amorphous solid; R_f 0.38 (6:3.5:0.5, CH_2Cl_2-methanol-H_2O); $[\alpha]_D$ -51 (*c* 0.85, H_2O); ^1H NMR (500 MHz, D_2O) δ 4.79 (d, 1 H, $J_{1',2'}$ 0.8 Hz, H-1′), 4.64 (d, 1 H, $J_{1,2}$ 0.6 Hz, H-1), 4.24 (dd, 1 H, $J_{1,2}$ 0.6, $J_{2,3}$ 3.1 Hz, H-2), 4.10 (dd, 1 H, $J_{1',2'}$ 0.8, $J_{2',3'}$ 3.4 Hz, H-2′), 3.94 (dd, 1 H, $J_{5',6'}$ 2.3, J_{gem} 12.4 Hz, H-6a′), 3.91 (dd, 1 H, $J_{5,6}$ 1.7, J_{gem} 12.5 Hz, H-6a), 3.71-3.77 (m, 3 H, H-6b′, H-3, H-6b), 3.64 (dd, 1 H, $J_{2',3'}$ 3.0, $J_{3',4'}$ 9.7 Hz, H-3′), 3.58 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.6 Hz, H-4′), 3.54 (s, 3 H, C*H*$_3$O-C4′), 3.53 (s, 3 H, C*H*$_3$O-C1), 3.39 (ddd, 1 H, $J_{4',5'}$ 9.5, $J_{5',6'}$ 2.2, 6.5 Hz, H-5′), 3.34-3.36 (m, 2 H, H-4, H-5); ^{13}C NMR (125 MHz, D_2O) δ 102.2 (C-1, $^1J_{C\text{-}1,H\text{-}1}$ 160.4 Hz, β), 101.3 (C-1′, $^1J_{C\text{-}1',H\text{-}1'}$ 162.6 Hz, β), 78.6 (C-2), 77.7 (C-4/C-5), 77.3 (C-5′), 76.2 (C-4/C-5), 73.4, 73.2 (C-3, C-3′), 71.4 (C-2′), 68.2 (C-4′), 61.8, 61.7 (C-6, C-6′), 61.0 (*C*H$_3$OC-4′), 58.0 (*C*H$_3$OC-1); ESI HRMS Calc'd. for $C_{14}H_{26}O_{11}Na$ 393.1367. Found 393.1366.



55

_p_-Methoxyphenyl 3,4-di-_O_-benzyl-β-D-glucopyranoside (55)

_p_-Methoxyphenyl 3-_O_-benzyl-4,6-_O_-benzylidene-β-D-glucopyranoside[207] **54** (750.0 mg, 1.62 mmol) was dissolved in dry CH_2Cl_2 (20 mL) under an argon atmosphere and the reaction was cooled to 0 °C (ice-water bath). A solution of 1.0 M BH_3·THF (8.1 mL, 8.08 mmol) was added followed by the dropwise addition of a solution of 1.0 M dibutylboron triflate in CH_2Cl_2 (808 μL, 0.81 mmol). The reaction was stirred at 0 °C for 4 h. The reaction was quenched with methanol, neutralized with Et_3N and concentrated under reduced pressure leaving a colourless syrup. Chromatography on silica gel (4:1, hexanes-EtOAc) gave **55** (649.4 mg, 86%) as a white amorphous solid; R_f 0.39 (1:1, hexanes-EtOAc); $[\alpha]_D$ -18 (_c_ 0.17, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30-7.41 (m, 10 H, ArH), 6.97-7.00 (m, 2 H, ArH), 6.83-6.85 (m, 2 H, ArH), 4.96 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.90 (m, 2 H, PhCH_2O), 4.83 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.69 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 3.89 (m, 1 H, H-6a), 3.78 (s, 3 H, CH_3O), 3.73-3.78 (m, 2 H, H-2, H-6b), 3.69 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 8.9 Hz, H-3), 3.65 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 8.9 Hz, H-4), 3.49 (ddd, 1 H, $J_{4,5}$ 9.2, $J_{5,6}$ 2.8, 4.7 Hz, H-5), 2.48 (bs, 1 H, C2-OH), 1.92 (bs, 1 H, C6-OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.5 (Ar), 151.0 (Ar), 138.5 (Ar), 137.9 (Ar), 128.5(3) (Ar), 128.5(2) (Ar), 128.1 (Ar), 127.9(8) (Ar), 127.9(6) (Ar), 127.8 (Ar), 118.2 (Ar), 114.7 (Ar), 101.8 (C-1), 84.2 (C-3), 77.2 (C-4), 75.6, 75.3, 75.1 (C-5, PhCH_2O×2), 74.5 (C-2), 62.0 (C-6), 55.7 (CH_3O); ESI HRMS Calc'd. for $C_{27}H_{30}O_7Na$ 489.1884. Found 489.1887.

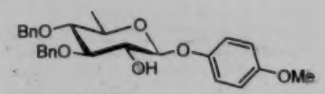


56

***p*-Methoxyphenyl 3,4-di-*O*-benzyl-6-*O*-methanesulfonyl-β-D-glucopyranoside (56)**

Compound **55** (618.1 mg, 1.32 mmol) was dissolved in dry pyridine (6 mL) then the solution was cooled to 0 °C under argon. A solution of methanesulfonyl chloride (113 μL, 1.46 mmol) in dry CH_2Cl_2 (6 mL) was added using a syringe pump (1.5 mL/h). The reaction was subsequently allowed to warm to room temperature. After stirring overnight, the reaction mixture was diluted with CH_2Cl_2 and washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, distilled water then brine. The organic phase was dried (Na_2SO_4) and then concentrated under reduced pressure. The clear syrup was chromatographed (7:3, hexanes-EtOAc) to yield **56** (692.5 mg, 96%); R_f 0.46 (1:1, hexanes-EtOAc); $[\alpha]_D$ –21 (*c* 0.43, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30-7.41 (m, 10 H, ArH), 6.97-7.00 (m, 2 H, ArH), 6.82-6.85 (m, 2 H, ArH), 4.99 (d, 1 H, J_{gem} 11.2 Hz, PhCH_2O), 4.93 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.89 (d, 1 H, J_{gem} 11.2 Hz, PhCH_2O), 4.80 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.66 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.47 (dd, 1 H, $J_{5,6}$ 2.1, J_{gem} 11.4 Hz, H-6a), 4.34 (dd, 1 H, $J_{5,6}$ 5.3, J_{gem} 11.4 Hz, H-6b), 3.76-3.80 (m, 4 H, H-2, CH_3O), 3.70 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 8.7 Hz, H-3), 3.66 (ddd, 1 H, $J_{4,5}$ 9.8, $J_{5,6}$ 2.2, 5.3 Hz, H-5), 3.58 (dd, 1 H, $J_{3,4}$ 8.7, $J_{4,5}$ 9.7 Hz, H-4), 2.92 (s, 3 H, CH_3O), 2.46 (d, 1 H, $J_{2,OH}$ 2.6 Hz, C2-OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.7 (Ar), 150.8 (Ar), 138.2 (Ar), 137.4 (Ar), 128.6(0) (Ar), 128.5(7) (Ar), 128.2 (Ar), 128.0 (Ar), 127.9 (Ar), 118.5 (Ar), 114.6 (Ar), 101.9 (C-1), 84.0 (C-3), 76.6 (C-4), 75.3 (PhCH_2O), 75.2 (PhCH_2O), 74.4 (C-2), 73.2 (C-5), 68.5 (C-6), 55.7 (CH_3O), 37.6 (CH_3SO_3); ESI HRMS Calc'd. for

$C_{28}H_{32}O_9SNa$ 567.1659. Found 567.1662. Anal. Calc'd. for $C_{28}H_{32}O_9S$: C, 61.75; H, 5.92; S, 5.89. Found: C, 61.59; H, 6.02; S, 5.83.

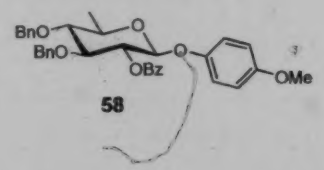


57

***p*-Methoxyphenyl 3,4-di-*O*-benzyl-6-deoxy-β-D-glucopyranoside (57)**

A solution of compound **56** (803.3 mg, 1.48 mmol) and sodium borohydride (111.6 mg, 2.95 mmol) were stirred in DMF (8 mL) at 80 °C. After 15 h, the reaction was concentrated under reduced pressure. The residue was redissolved in EtOAc then washed with 2% aqueous citric acid, saturated aqueous $NaHCO_3$, distilled water then brine. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. Purification by chromatography (98:2, toluene-EtOAc) gave a white solid **57** (664.6 mg, 99%); R_f 0.61 (1:1, hexanes-EtOAc); $[\alpha]_D$ -15 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29-7.41 (m, 10 H, ArH), 6.99-7.01 (m, 2 H, ArH), 6.82-6.85 (m, 2 H, ArH), 4.96 (d, 1 H, J_{gem} 11.4 Hz, PhCH_2O), 4.92 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.89 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.76 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.68 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 3.78 (m, 1 H, H-2), 3.78 (s, 3 H, CH_3O), 3.63 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 9.0 Hz, H-3), 3.53 (dq, 1 H, $J_{4,5}$ 9.4, $J_{5,6}$ 6.2 Hz, H-5), 3.29 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.1 Hz, H-4), 2.42 (d, 1 H, $J_{2,OH}$ 2.5 Hz, OH), 1.36 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.4 (Ar), 151.2 (Ar), 138.6 (Ar), 138.1 (Ar), 128.5(0) (Ar), 128.4(6) (Ar), 128.0(2) (Ar), 127.9(7) (Ar), 127.8(7) (Ar), 127.8(0) (Ar), 118.4 (Ar), 114.6 (Ar), 101.8 (C-1), 84.2 (C-3), 83.0 (C-4), 75.4 (Ph*C*H$_2$O), 75.2 (Ph*C*H$_2$O), 74.8 (C-2), 71.7 (C-5), 55.6

(CH_3O), 18.0 (C-6); ESI HRMS Calc'd. for $C_{27}H_{30}O_6Na$ 473.1935. Found 472.1935. Anal. Calc'd. for $C_{27}H_{30}O_6$: C, 71.98; H, 6.71. Found: C, 71.94; H, 6.69.

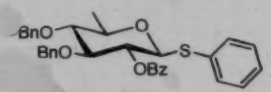


58

***p*-Methoxyphenyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-6-deoxy-β-D-glucopyranoside (58)**

Compound **57** (365.6 mg, 0.81 mmol) was dissolved in pyridine (10 mL) and benzoyl chloride (188 μL, 1.62 mmol) was added. The reaction was stirred at room temperature overnight then concentrated under reduced pressure. The residue was redissolved in EtOAc then washed with 10% aqueous HCl, saturated aqueous $NaHCO_3$, distilled water then brine. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. Purification by chromatography (98:2, toluene-EtOAc) gave a white solid **58** (430.0 mg, 96%); R_f 0.64 (1:1, hexanes-EtOAc); $[\alpha]_D$ +44 (*c* 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 8.01-8.03 (m, 2 H, ArH), 7.57 (m, 1 H, ArH), 7.42-7.45 (m, 2 H, ArH), 7.30-7.37 (m, 5 H, ArH), 7.13-7.17 (m, 5 H, ArH), 6.88-6.90 (m, 2 H, ArH), 6.74-6.77 (m, 2 H, ArH), 5.49 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.6 Hz, H-2), 4.96 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.91 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.77 (d, 1 H, J_{gem} 11.1 Hz, PhCH_2O), 4.70 (m, 2 H, PhCH_2O), 3.85 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 9.1 Hz, H-3), 3.73 (s, 3 H, CH_3O), 3.60 (dq, 1 H, $J_{4,5}$ 9.3, $J_{5,6}$ 6.2 Hz, H-5), 3.45 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.0 Hz, H-4), 1.41 (d, 3 H, $J_{5,6}$ 6.2 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2 (*C*=O), 155.4 (Ar), 151.5 (Ar), 137.9 (Ar), 137.7 (Ar), 133.1 (Ar), 129.9 (Ar), 129.8 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.7 (Ar), 118.7 (Ar), 114.4 (Ar), 100.7 (C-1), 83.3 (C-4), 82.6 (C-3), 75.4 (PhCH_2O), 75.1 (PhCH_2O), 74.0 (C-2), 71.7 (C-5), 55.6 (CH_3O),

18.0 (C-6); ESI HRMS Calc'd. for $C_{34}H_{34}O_7Na$ 577.2197. Found 577.2198; Anal. Calc'd. for $C_{34}H_{34}O_7$: C, 73.63; H, 6.18. Found: C, 73.64; H, 6.17.

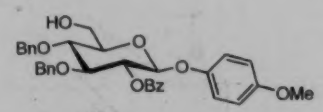


59

Phenyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-6-deoxy-1-thio-β-D-glucopyranoside (59)

Compound **58** (600 mg, 1.08 mmol) was mixed with thiophenol (222 μL, 2.16 mmol) in dry CH_2Cl_2 (10 mL). $BF_3{\cdot}OEt_2$ (165 μL, 1.30 mmol) was added dropwise and the reaction was stirred at 0 °C (ice-water bath) under argon. After 3 h, the reaction was quenched with Et_3N and diluted with CH_2Cl_2. The organic phase was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4) and concentrated under reduced pressure. Purification of the crude product by chromatography over silica gel (98:2, toluene-EtOAc) yielded **59** (500 mg, 86%) as a white crystalline solid; R_f 0.71 (1:1, hexanes-EtOAc); $[\alpha]_D$ +51 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03-8.06 (m, 2 H, ArH), 7.59 (m, 1 H, ArH), 7.43-7.47 (m, 4 H, ArH), 7.26-7.37 (m, 7 H, ArH), 7.10-7.14 (m, 5 H, ArH), 5.27 (dd, 1 H, $J_{1,2}$ 10.1, $J_{2,3}$ 9.3 Hz, H-2), 4.87 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.78 (d, 1 H, $J_{1,2}$ 10.1 Hz, H-1), 4.73 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.63-4.68 (m, 2 H, PhCH_2O), 3.81 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 9.0 Hz, H-3), 3.54 (dq, 1 H, $J_{4,5}$ 9.3, $J_{5,6}$ 6.1 Hz, H-5), 3.35 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.2 Hz, H-4), 1.40 (d, 3 H, $J_{5,6}$ 6.1 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2 (*C*=O), 137.9 (Ar), 137.7 (Ar), 133.1(4) (Ar), 133.0(9) (Ar), 132.4 (Ar), 130.0 (Ar), 129.8 (Ar), 128.8 (Ar), 128.5 (Ar), 128.4 (Ar), 128.2 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 86.2 (C-1), 84.2 (C-3), 83.2 (C-4), 75.9,

75.4, 75.3 (C-5, $PhCH_2O\times2$), 72.8 (C-2), 18.2 (C-6); ESI HRMS Calc'd. for $C_{33}H_{32}O_5SNa$ 563.1863. Found 563.1862. Anal. Calc'd. for $C_{33}H_{34}O_5S$: C, 73.31; H, 5.97; S, 5.93. Found: C, 73.31; H, 5.92; S, 5.57.

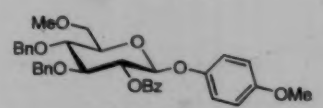


60

***p*-Methoxyphenyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-glucopyranoside (60)**

p-Methoxyphenyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-*β*-D-gluco-pyranoside[205] **41** (2.00 g, 3.52 mmol) was dissolved in dry CH_2Cl_2 (20 mL) and the solution was cooled to 0 °C in an ice bath under argon. A solution of 1.0 M BH_3·THF (5.8 mL, 5.82 mmol) was added followed by the dropwise addition of a solution of 1.0 M dibutylboron triflate in CH_2Cl_2 (580 μL, 0.58 mmol). The reaction was stirred at 0 °C for 4 h. The reaction was quenched with methanol and neutralized by addition of Et_3N. The reaction mixture was concentrated under reduced pressure leaving a clear syrup. Chromatography in hexanes:EtOAc (4:1) gave **60** (1.91 g, 95%) as a white amorphous solid; R_f 0.49 (1:1, hexanes-EtOAc); $[\alpha]_D$ +36 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02-8.04 (m, 2 H, ArH), 7.58 (m, 1 H, ArH), 7.42-7.46 (m, 2 H, ArH), 7.30-7.38 (m, 5 H, ArH), 7.14-7.18 (m, 5 H, ArH), 6.86-6.89 (m, 2 H, ArH), 6.74-6.77 (m, 2 H, ArH), 5.49 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.3 Hz, H-2), 5.05 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.90 (d, 1 H, J_{gem} 11.0 Hz, $PhCH_2O$), 4.79 (d, 1 H, J_{gem} 11.2 Hz, $PhCH_2O$), 4.71 (m, 2 H, $PhCH_2O$), 3.95 (ddd, 1 H, $J_{5,6}$ 2.7, J_{gem} 12.2, $J_{6,OH}$ 5.9 Hz, H-6a), 3.92 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 9.0 Hz, H-3), 3.83 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.4 Hz, H-4), 3.79 (ddd, 1 H, $J_{5,6}$ 4.5, $J_{6,OH}$ 7.7, J_{gem}

12.2 Hz, H-6b), 3.74 (s, 3 H, CH_3O), 3.58 (ddd, 1 H, $J_{4,5}$ 9.6, $J_{5,6}$ 2.7, 4.7 Hz, H-5), 1.95 (dd, 1 H, $J_{6,OH}$ 6.0, 7.9 Hz, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2 (C=O), 155.5 (Ar), 151.2 (Ar), 137.7 (Ar), 137.6 (Ar), 133.2 (Ar), 129.8 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.1 (Ar), 128.0 (Ar), 127.7 (Ar), 118.4 (Ar), 114.6 (Ar), 100.7 (C-1), 82.5 (C-3), 77.5 (C-4), 75.7 (C-5), 75.2 ($PhCH_2O$), 75.1 ($PhCH_2O$), 73.6 (C-2), 61.9 (C-6), 55.6 (CH_3O); ESI HRMS Calc'd. for $C_{30}H_{34}O_8Na$ 593.2146. Found 593.2148; Anal. Calc'd. for $C_{34}H_{34}O_8$: C, 71.56; H, 6.01. Found: C, 71.22; H, 6.19.

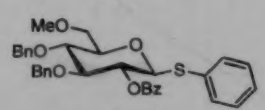


61

***p*-Methoxyphenyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-methyl-β-D-glucopyranoside (61)**

To a solution of compound **60** (800 mg, 1.40 mmol) in dry DMF (20 mL) was added methyl iodide (175 μL, 2.80 mmol). The reaction was cooled to 0 °C (ice-water bath) under argon before the addition of sodium hydride (112 mg, 2.80 mmol). After 3 hours stirring at 0 °C, the reaction was quenched with acetic acid then concentrated under reduced pressure. Purification by chromatography over silica gel (7:3, hexanes-EtOAc) gave **61** (654 mg, 80%) as a white solid; R_f 0.59 (1:1, hexanes-EtOAc); $[\alpha]_D$ +30 (*c* 1.0 $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.01-8.03 (m, 2 H, ArH), 7.57 (m, 1 H, ArH), 7.42-7.45 (m, 2 H, ArH), 7.30-7.38 (m, 5 H, ArH), 7.12-7.17 (m, 5 H, ArH), 6.89-6.91 (m, 2 H, ArH), 6.74-6.77 (m, 2 H, ArH), 5.51 (dd, 1 H, $J_{1,2} \approx J_{2,3}$ 8.6 Hz, H-2), 4.97 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.89 (d, 1 H, J_{gem} 11.0 Hz, $PhCH_2O$), 4.78 (d, 1 H, J_{gem} 11.3 Hz,

PhCH_2O), 4.70 (d, 1 H, J_{gem} 11.2 Hz, PhCH_2O), 4.69 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 3.89 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 9.0 Hz, H-3), 3.85 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.0 Hz, H-4), 3.73 (s, 3 H, CH_3O), 3.66-3.72 (m, 2 H, H-6a, H-6b), 3.62 (ddd, 1 H, $J_{4,5}$ 8.9, $J_{5,6}$ 1.8, 3.9 Hz, H-5), 3.42 (s, 3 H, CH_3O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2 (*C*=O), 155.4 (Ar), 151.6 (Ar), 138.0 (Ar), 137.8 (Ar), 133.1 (Ar), 129.9 (Ar), 129.8 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 127.6 (Ar), 118.7 (Ar), 114.4 (Ar), 101.0 (C-1), 82.6 (C-3), 77.8 (C-4), 75.3 (C-5), 75.1 (PhCH_2O), 75.0 (PhCH_2O), 73.7 (C-2), 71.1 (C-6), 59.5 (*C*H_3O), 55.6 (*C*H_3O); ESI HRMS Calc'd. for $C_{35}H_{36}O_8Na$ 607.2302. Found 607.2308. Anal. Calc'd. for $C_{35}H_{36}O_8$: C, 71.90; H, 6.21. Found: C, 72.14; H, 6.20.

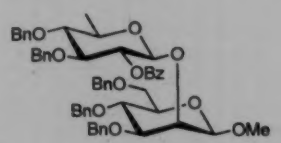


62

Phenyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-methyl-1-thio-β-D-glucopyranoside (62)

Thiophenol (56 μL, 0.54 mmol) was added to a solution of **61** (158.2 mg, 0.27 mmol) in dry CH_2Cl_2 (8 mL) under argon. The mixture was cooled to 0 °C in an ice bath. $BF_3{\cdot}OEt_2$ (41 μL, 0.32 mmol) was added and the reaction was stirred at 0 °C for 3 h. The reaction was neutralized with Et_3N, diluted with CH_2Cl_2 and washed following the general protocol. The organic phase was dried (Na_2SO_4) then concentrated under reduced pressure. Chromatography over silica gel with toluene:EtOAc (95:5) gave a white solid **62** (135.5 mg, 88%); R_f 0.67 (1:1, hexanes-EtOAc); $[\alpha]_D$ +52 (*c* 0.36, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03-8.05 (m, 2 H, ArH), 7.59 (m, 1 H, ArH), 7.44-7.48 (m, 4 H, ArH), 7.25-7.37 (m, 8 H, ArH), 7.11-7.14 (m, 5 H, ArH), 5.29 (dd, 1 H, $J_{1,2}$ 10.0, $J_{2,3}$ 9.2

Hz, H-2), 4.85 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.78 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 4.74 (d, 1 H, J_{gem} 11.1 Hz, PhCH_2O), 4.65-4.67 (m, 2 H, PhCH_2O), 3.85 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 9.1 Hz, H-3), 3.75 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.6 Hz, H-4), 3.70 (dd, 1 H, $J_{5,6}$ 2.1, J_{gem} 11.0 Hz, H-6a), 3.67 (dd, 1 H, $J_{5,6}$ 4.4, J_{gem} 11.0 Hz, H-6b), 3.56 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6}$ 2.1, 4.4 Hz, H-5), 3.41 (s, 3 H, CH_3O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2 (*C*=O), 138.0 (Ar), 137.7 (Ar), 133.3 (Ar), 133.1 (Ar), 132.3 (Ar), 129.9(3) (Ar), 129.8(6) (Ar), 128.8 (Ar), 128.5 (Ar), 128.4 (Ar), 128.2 (Ar), 127.9(9) (Ar), 127.9(8) (Ar), 127.8(8) (Ar), 127.7 (Ar), 127.6 (Ar), 86.5 (C-1), 84.2 (C-3), 79.4 (C-5), 77.7 (C-4), 75.3 (PhCH_2O), 75.1 (PhCH_2O), 72.6 (C-2), 71.3 (C-6), 59.5 (CH_3O); ESI HRMS Calc'd. for $C_{34}H_{34}O_6SNa$ 593.1968. Found 593.1967. Anal. Calc'd. for $C_{34}H_{34}O_6S$: C, 71.56; H, 6.00; S, 5.62. Found: C, 71.50; H, 6.08; S, 5.66.

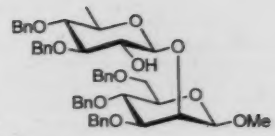


63

Methyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-6-deoxy-β-D-glucopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (63)

Compound **59** (250 mg, 0.46 mmol) and methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside[183] **20** (236 mg, 0.51 mmol) in dry CH_2Cl_2 (5 mL) were reacted with N-iodosuccinimide (125 mg, 0.55 mmol) and silver triflate (24 mg, 0.09 mmol) then processed as described for **48**. The product was purified by column chromatography over silica gel (95:5, toluene-EtOAc) to give **63** (339 mg, 82%); R_f 0.62 (1:1, hexanes-EtOAc);

$[\alpha]_D$ -37 (*c* 0.45, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.05-8.07 (m, 2 H, ArH), 7.13-7.47 (m, 28 H, ArH), 5.40 (dd, 1 H, $J_{1',2'}$ 8.1, $J_{2',3'}$ 9.6 Hz, H-2′), 4.96 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.92 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.90 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.83 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.75 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.71 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.68 (d, 1 H, J_{gem} 11.1 Hz, PhCH_2O), 4.60 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.48 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.18 (d, 1 H, J_{gem} 11.8 Hz, PhCH_2O), 4.17 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 4.12 (s, 1 H, H-1), 4.05 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 3.86 (dd, 1 H, $J_{2',3'} \approx J_{3',4'}$ 9.2 Hz, H-3′), 3.66 (dd, 1 H, $J_{5,6}$ 1.4, J_{gem} 10.9 Hz, H-6a), 3.59 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.1 Hz, H-4), 3.53 (dq, 1 H, $J_{4',5'}$ 9.3, $J_{5',6'}$ 6.3 Hz, H-5′), 3.42-3.46 (m, 2 H, H-4′, H-3), 3.30 (ddd, 1 H, $J_{4,5}$ 9.4, $J_{5,6}$ 1.4, 7.1 Hz, H-5), 3.26 (s, 3 H, CH_3O), 3.24 (dd, 1 H, $J_{5,6}$ 7.1, J_{gem} 10.9 H-6b), 1.40 (d, 3 H, $J_{5',6'}$ 6.0 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.3 (*C*=O), 138.7 (Ar), 138.5 (Ar), 138.2(1) (Ar), 138.1(9) (Ar), 132.4 (Ar), 131.0 (Ar), 130.0 (Ar), 128.4 (Ar), 128.3(4) (Ar), 128.2(7) (Ar), 128.1(8) (Ar), 128.1(3) (Ar), 128.1(1) (Ar), 128.0(3) (Ar), 127.9(7) (Ar), 127.9 (Ar), 127.8 (Ar), 127.6 (Ar), 127.5(1) (Ar), 127.4(9) (Ar), 127.3 (Ar), 101.5 (C-1), 101.0 (C-1′), 83.1 (C-4′/C-3), 82.9 (C-3′), 80.5 (C-4′/C-3), 76.2 (C-5), 75.2 (×2), 74.9, 74.8 (C-4, PhCH_2O×3), 74.3 (C-2′), 73.3 (PhCH_2O), 72.9 (C-2), 71.3 (C-5), 70.8 (PhCH_2O), 70.4 (C-6), 56.6 (CH_3O), 18.0 (C-6′); ESI HRMS Calc'd. for $C_{55}H_{58}O_{11}Na$ 917.3871. Found 917.3885; Anal. Calc'd. for $C_{55}H_{58}O_{11}$: C, 73.81; H, 6.53. Found: C, 73.44; H, 6.56.

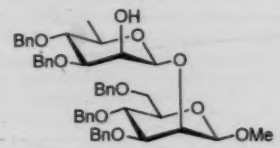


64

Methyl 3,4-di-*O*-benzyl-6-deoxy-β-D-glucopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (64)

Disaccharide **63** (273 mg, 0.31 mmol) was dissolved in CH_2Cl_2-methanol (1:1, 10 mL) and treated with 0.5 M MeONa/MeOH (2 mL) then processed as described for **23**. Purification of the product by chromatography over silica gel (1:1, hexanes-EtOAc) gave **64** (160 mg, 67%) as a clear syrup; R_f 0.30 (1:1, hexanes-EtOAc); $[\alpha]_D$ -37 (*c* 0.35, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41-7.43 (m,4 H, ArH), 7.26-7.38 (m, 19 H, ArH), 7.20-7.21 (m, 2 H, ArH), 5.07 (d, 1 H, J_{gem} 11.2 Hz, PhCH_2O), 4.93 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.92 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.84 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.81 (d, 1 H, J_{gem} 11.2 Hz, PhCH_2O), 4.65 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.63 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.62 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.58 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.57 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.51 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.31 (d, 1 H, $J_{1,2}$ 0.4 Hz, H-1), 4.17 (d, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 3.90 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4), 3.80 (dd, 1 H, $J_{5,6}$ 2.1, J_{gem} 11.0 Hz, H-6a), 3.76 (dd, 1 H, $J_{5,6}$ 5.0, J_{gem} 11.3 Hz, H-6b), 3.74 (dd, 1 H, $J_{1',2'}$ 7.8, $J_{2',3'}$ 8.9 Hz, H-2′), 3.64 (dd, 1 H, $J_{2',3'} \approx J_{3',4'}$ 8.9 Hz, H-3′), 3.55 (s, 3 H, CH_3O), 3.54 (dd, 1 H, H-3), 3.42-3.47 (m, 3 H, H-5′, H-5, OH), 3.28 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.1 Hz, H-4′), 1.34 (d, 3 H, $J_{5',6'}$ 6.2 Hz, H-6′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.2 (Ar), 138.4 (Ar), 138.3 (Ar), 138.0 (Ar), 128.3(5) (Ar), 128.3(4) (Ar), 128.3(3) (Ar), 128.3(1) (Ar), 128.2 (Ar), 128.1 (Ar), 128.0(2) (Ar),

128.0(1) (Ar), 127.9(6) (Ar), 127.7(0) (Ar), 127.6(9) (Ar), 127.6(4) (Ar), 127.5(6) (Ar), 127.4 (Ar), 104.5 (C-1′), 101.5 (C-1), 85.1 (C-3′), 82.3 (C-4′), 80.7 (C-3), 75.7(8), 75.7(5) (C-2′, C-5), 75.4, 75.3, 75.2 (C-2, Ph*C*H_2O×2), 74.7, 74.5 (C-4, Ph*C*H_2O), 73.5 (Ph*C*H_2O), 71.6 (C-5′), 71.1 (Ph*C*H_2O), 69.2 (C-6), 57.1 (*C*H_3O), 18.0 (C-6′); ESI HRMS Calc'd. for $C_{48}H_{54}O_{10}Na$ 813.3609. Found 813.3610; Anal. Calc'd. for $C_{48}H_{54}O_{10}$: C, 72.89; H, 6.88. Found: C, 73.04; H, 6.95.

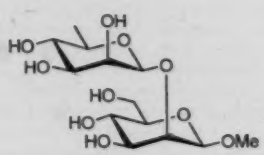


65

Methyl 3,4-di-*O*-benzyl-6-deoxy-β-D-mannopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (65)

Same procedure as described for compound **24**. Disaccharide **64** (133 mg, 0.17 mmol) was stirred in a mixture of Me_2SO (5 mL) and acetic anhydride (2.5 mL). Following concentration under reduced pressure the residue was reacted with L-Selectride® (1 ml, 1.0 M solution in THF) in dry THF (5 mL) at -78 °C. Purification of the product by chromatography over silica gel (1:1, hexanes-EtOAc) gave **65** (98 mg, 73%) as a colourless syrup; R_f 0.45 (1:1, hexanes-EtOAc); $[\alpha]_D$ -52 (*c* 1.03, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39-7.42 (m, 4 H, ArH), 7.25-7.39 (m, 19 H, ArH), 7.15-7.17 (m, 2 H, ArH), 5.01 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.86-4.88 (m, 2 H, PhCH_2O), 4.84 (d, 1 H, $J_{1',2'}$ 0.8 Hz, H-1′), 4.84 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O), 4.62-4.67 (m, 2 H, PhCH_2O), 4.64 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.55 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O),

4.50 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.48 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.43 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 4.32 (dd, 1 H, $J_{1',2'}$ 0.8, $J_{2',3'}$ 2.8 Hz, H-2′), 4.29 (d, 1 H, $J_{1,2}$ 0.6 Hz, H-1), 3.80 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4), 3.79 (dd, 1 H, $J_{5,6}$ 1.9, J_{gem} 10.8 Hz, H-6a), 3.73 (dd, 1 H, $J_{5,6}$ 5.5, J_{gem} 10.7 Hz, H-6b), 3.64 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.2 Hz, H-4′), 3.55 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 9.2 Hz, H-3), 3.51 (dd, 1 H, $J_{2',3'}$ 2.9, $J_{3',4'}$ 9.2 Hz, H-3′), 3.50 (s, 3 H, CH_3O), 3.43 (ddd, 1 H, $J_{4,5}$ 9.6, $J_{5,6}$ 1.8, 5.4 Hz, H-5), 3.36 (dq, 1 H, $J_{4',5'}$ 9.3, $J_{5',6'}$ 6.1 Hz, H-5′), 1.35 (d, 3 H, $J_{5',6'}$ 6.1 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.6 (Ar), 138.3 (Ar), 138.2(5) (Ar), 138.1(9) (Ar), 138.1 (Ar), 128.3(5) (Ar), 128.3(3) (Ar), 128.3(1) (Ar), 128.2(9) (Ar), 128.1(2) (Ar), 128.1(0) (Ar), 127.9(1) (Ar), 127.9(0) (Ar), 127.6(3) (Ar), 127.6(1) (Ar), 127.5(9) (Ar), 127.5(8) (Ar), 102.1 (C-1), 99.1 (C-1′), 81.2 (C-3′), 80.5 (C-3), 79.6 (C-4′), 75.6 (C-5), 75.4 (PhCH_2O), 75.1 (PhCH_2O), 74.2 (C-4), 73.5 (PhCH_2O), 71.5 (C-5′), 70.7, 70.6, 70.5 (C-2, PhCH_2O×2), 69.4 (C-6), 67.9 (C-2′), 57.2 (CH_3O), 18.1 (C-6′); ESI HRMS Calc'd. for $C_{48}H_{54}O_{10}Na$ 813.3609. Found 813.3616.

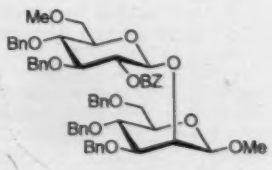


10

Methyl 6-deoxy-β-D-mannopyranosyl (1→2) β-D-mannopyranoside (10)

Compound **65** (48.1 mg, 0.061 mmol) was dissolved in ethanol (5 mL)-ethyl acetate (5 mL) and stirred with 10% Pd/C (50 mg) under an H_2 atmosphere then processed as described for **1**. Filtration then lyophilization gave **10** (13.5 mg, 65%) as a clear glass; R_f 0.32 (6:3.5:0.5, CH_2Cl_2-MeOH-H_2O); $[\alpha]_D$ -72 (*c* 0.30, H_2O); ^{1}H NMR

(600 MHz, D_2O) δ 4.75 (s, 1 H, H-1′), 4.65 (s, 1 H, H-1), (d, 1 H, $J_{1,2}$ Hz, H-2), (d, 1 H, $J_{1',2'}$ Hz, H-2′), (dd, 1 H, $J_{5,6}$, J_{gem} Hz, H-6a), (dd, 1 H, $J_{5,6}$ Hz, H-6b), (m, 3 H, H-3′, H-3, H-4), (s, 3 H, CH_3O), (m, 3 H, H-4′, H-5′, H-5), (d, 3 H, $J_{5',6'}$ Hz, H-6′); ^{13}C NMR (125 MHz, D_2O) δ 102.1 (C-1, $^1J_{C\text{-}1,H\text{-}1}$ 160.0 Hz, β), 101.5 (C-1′, $^1J_{C\text{-}1',H\text{-}1'}$ 160.9 Hz, β), 79.3 (C-2), 77.3 (C-4′/C-5′/C-5), 73.3, 73.2, 73.1, 72.8 (C-3′, C-3, C-4′/C-5′/C-5), 71.3 (C-2′), 68.3 (C-4′), 61.8 (C-6), 58.0 (CH_3O), 17.6 (C-6′); ESI HRMS Calc'd. for $C_{13}H_{24}O_{10}Na$ 363.1262. Found 363.1264.

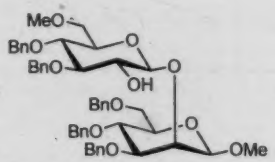


66

Methyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-methyl-β-D-glucopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (66)

Compound **62** (135.5 mg, 0.237 mmol) and methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside[183] **20** (132.3 mg, 0.285 mmol) in dry CH_2Cl_2 (5 mL) were reacted with N-iodosuccinimide (64.1 mg, 0.285 mmol) and trifluoromethane sulfonic acid (7 μL) then processed as described for **48**. The product was purified by column chromatography over silica gel (95:5, toluene-EtOAc) to give **66** (186.3 mg, 85%) as a clear syrup; R_f 0.55 (1:1, hexanes-EtOAc); $[\alpha]_D$ -34 (*c* 0.58, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05-8.06 (m, 2 H, ArH), 7.13-7.47 (m, 28 H, ArH), 5.39 (dd, 1 H, $J_{1',2'}$ 8.1, $J_{2',3'}$ 9.4 Hz, H-2′), 4.99 (d, 1 H, H-1′), 4.89-4.93 (m, 2 H, PhCH_2O), 4.89 (d, 1 H, J_{gem} 11.1 Hz, PhCH_2O), 4.75 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.72 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.64 (d, 1 H, J_{gem}

11.2 Hz, PhCH_2O), 4.56 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.47 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.26 (d, 1 H, $J_{2,3}$ 2.9 Hz, H-2), 4.18 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.13 (s, 1 H, H-1), 4.06 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 3.91 (dd, 1 H, $J_{2',3'} \approx J_{3',4'}$ 8.8 Hz, H-3′), 3.56-3.70 (m, 6 H, H-4′, H-5′ H-6a′, H-6b′, H-4, H-6a), 3.45 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3), 3.33 (s, 3 H, CH_3O), 3.30 (ddd, 1 H, J 1.5, 7.1 Hz, H-5), 3.27 (s, 3 H, CH_3O), 3.24 (dd, 1 H, $J_{5,6}$ 7.1, J_{gem} 10.9 Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.3 (*C*=O), 138.7 (Ar), 138.6 (Ar), 138.4 (Ar), 138.1 (Ar), 132.4 (Ar), 130.9 (Ar), 130.0 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 128.1 (Ar), 128.0 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.6 (Ar), 127.5 (Ar), 127.5 (Ar), 127.3 (Ar), 101.6 (C-1), 101.1 (C-1′), 83.0 (C-3′), 80.2 (C-3), 78.1, 76.2, 75.2, 74.9, 74.8, 74.0, 73.3, 72.5, 72.1 (C-5′, C-4′, C-2′, C-6, C-5, C-4, C-2, Ph*C*H_2O×4), 70.4 (C-6′), 70.1 (Ph*C*H_2O), 59.1 (*C*H_3O), 56.6 (*C*H_3O); ESI HRMS Calc'd. for $C_{56}H_{60}O_{12}Na$ 947.3977. Found 947.3976.

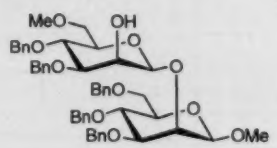


67

Methyl 3,4-di-*O*-benzyl-6-*O*-methyl-β-D-glucopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (67)

Disaccharide **66** (125.1 mg, 0.14 mmol) was dissolved in CH_2Cl_2-methanol (1:1, 4 mL) and treated with 0.5 M MeONa/MeOH (1 mL) then processed as described for **23**. Purification of the product by chromatography over silica gel (7:3, hexanes-EtOAc) gave **67** (94.9 mg, 85%) as a clear syrup; R_f 0.32 (1:1, hexanes-EtOAc); $[\alpha]_D$ -32 (*c* 0.23,

$CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.27-7.43 (m, 23 H, ArH), 7.18-7.20 (m, 2 H, ArH), 5.07 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.89-4.94 (m, 3 H, PhCH_2O×3), 4.80 (d, 1 H, J_{gem} 11.4 Hz, PhCH_2O), 4.55-4.64 (m, 5 H, H-1′, PhCH_2O×4), 4.49 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.30 (s, 1 H, H-1), 4.24 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 3.89 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4), 3.66-3.80 (m, 4 H, H-2′, H-3′, H-6a, H-6b), 3.50-3.60 (m, 5 H, H-4′, H-5′, H-6a′, H-6b′, H-3), 3.54 (s, 3 H, CH_3O), 3.41-3.44 (m, 2 H, H-5, OH), 3.29 (s, 3 H, CH_3O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.1 (Ar), 138.4(4) (Ar), 138.3(7) (Ar), 138.3 (Ar), 138.2 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.0(4) (Ar), 128.0(2) (Ar), 127.9 (Ar), 127.7 (Ar), 127.6(1) (Ar), 127.5(6) (Ar), 127.4 (Ar), 104.4 (C-1′), 101.5 (C-1), 85.2 (C-3′), 80.5 (C-3), 75.7, 75.5 (C-5, PhCH_2O), 75.2, 75.1, 75.0, 74.9, 74.7 (C-2′, C-4′, C-2, PhCH_2O×2), 74.4 (C-4), 73.4 (PhCH_2O), 72.0 (C-6′), 70.6 (PhCH_2O), 69.2 (C-6), 59.0 (CH_3O), 57.1 (CH_3O); ESI HRMS Calc'd. for $C_{49}H_{56}O_{11}Na$ 843.3715. Found 843.3717; Anal. Calc'd. for $C_{49}H_{56}O_{11}$: C, 71.69; H, 6.88. Found: C, 71.60; H, 6.86.

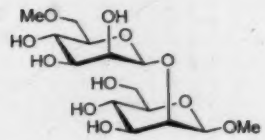


68

Methyl 3,4-di-*O*-benzyl-6-*O*-methyl-β-D-mannopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (68)

Same procedure as described for compound **24**. Disaccharide **67** (121.1 mg, 0.15 mmol) was stirred in a mixture of Me_2SO (2 mL) and acetic anhydride (1 mL). Following concentration under reduced pressure the residue was reacted with L-Selectride® (0.5 ml, 1.0 M solution in THF) in dry THF (1 mL) at -78 °C. Purification of

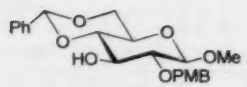
the product by chromatography over silica gel (1:1, hexanes-EtOAc) gave **68** (48 mg, 60%) as a colourless syrup; R_f 0.52 (1:1, hexanes-EtOAc); $[\alpha]_D$ -13 (*c* 0.34, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.26-7.44 (m, 23 H, ArH), 7.16-7.17 (m, 2 H, ArH), 4.99 (d, 1 H, J_{gem} 11.1 Hz, PhCH_2O), 4.94 (d, 1 H, J_{gem} 11.7 Hz, PhCH_2O), 4.89 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.88 (d, 1 H, $J_{1',2'}$ 0.9 Hz, H-1′), 4.85 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.65 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.61-4.65 (m, 2 H, PhCH_2O), 4.56 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.51 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.49 (d, 1 H, $J_{2,3}$ 2.9 Hz, H-2), 4.48 (d, 1 H, J_{gem} 10.5 Hz, PhCH_2O), 4.34 (d, 1 H, $J_{2',3'}$ 3.0 Hz, H-2′), 4.29 (s, 1 H, H-1), 3.88 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.4 Hz, H-4′), 3.80 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4), 3.79 (dd, 1 H, $J_{5,6}$ 1.9, J_{gem} 10.8 Hz, H-6a), 3.73 (dd, 1 H, $J_{5,6}$ 5.4, J_{gem} 10.8 Hz, H-6b), 3.64 (dd, 1 H, $J_{5',6'}$ 2.0, J_{gem} 10.6 Hz, H-6a′), 3.55-3.59 (m, 2 H, H-3′, H-3), 3.54 (dd, 1 H, $J_{5',6'}$ 6.3, J_{gem} 10.4 Hz, H-6b′), 3.50 (s, 3 H, CH_3O), 3.42-3.46 (m, 2 H, H-5′, H-5), 3.28 (s, 3 H, CH_3O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.6 (Ar), 138.4 (Ar), 138.3(0) (Ar), 138.2(8) (Ar), 138.2 (Ar), 128.3(6) (Ar), 128.3(5) (Ar), 128.2(9) (Ar), 128.2(7) (Ar), 128.1(3) (Ar), 128.1(0) (Ar), 128.0 (Ar), 127.9(1) (Ar), 127.8(9) (Ar), 127.7 (Ar), 127.6(0) (Ar), 127.5(8) (Ar), 127.5 (Ar), 102.1 (C-1), 99.2 (C-1′), 81.4 (C-3′), 80.4 (C-3), 75.6 (C-5), 75.1(1), 75.1(0), 74.9 (C-5′, PhCH_2O×2), 74.5 (C-4′), 74.1 (C-4), 73.5 (PhCH_2O), 72.3 (C-6′), 70.7, 70.6 (C-2, PhCH_2O), 70.1 (PhCH_2O), 69.4 (C-6), 67.7 (C-2′), 59.0 (CH_3O), 57.2 (CH_3O); ESI HRMS Calc'd. for $C_{49}H_{56}O_{11}Na$ 843.3715. Found 843.3716.



11

Methyl 6-*O*-methyl-β-D-mannopyranosyl (1→2) β-D-mannopyranoside (11)

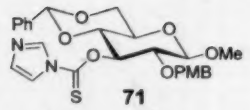
Compound **68** (70.0 mg, 0.085 mmol) was dissolved in EtOAc (5 mL)-methanol (5 mL) and stirred with 10% Pd/C (70 mg) under a H_2 atmosphere then processed as described for **1**. Filtration then lyophilization gave **11** (31.2 mg, 99%); R_f 0.34 (6:3.5:0.5, CH_2Cl_2-MeOH-H_2O); $[\alpha]_D$ -61 (*c* 0.77, H_2O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.82 (s, 1 H, $J_{1',2'}$ 0.7 Hz, H-1′), 4.66 (s, 1 H, $J_{1,2}$ 0.6 Hz, H-1), 4.25 (dd, 1 H, $J_{2,3}$ 2.6 Hz, H-2), 4.13 (dd, 1 H, $J_{1',2'}$ 0.6, $J_{2',3'}$ 3.2 Hz, H-2′), 3.95 (dd, 1 H, $J_{5,6}$ 2.2, J_{gem} 12.3 Hz, H-6a), 3.79 (dd, 1 H, $J_{5',6'}$ 2.1, J_{gem} 11.2 Hz, H-6a′), 3.75 (dd, 1 H, $J_{5,6}$ 6.5, J_{gem} 12.3 Hz, H-6b), 3.68 (dd, 1 H, $J_{5',6'}$ 6.8, J_{gem} 11.3 Hz, H-6b′), 3.63-3.60 (m, 2 H, H-3′, H-3), 3.58 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4), 3.54 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.7 Hz, H-4′), 3.55 (s, 3 H, C*H*$_3$O-C1), 3.49 (ddd, 1 H, $J_{4',5'}$ 9.8, $J_{5',6'}$ 2.0, 6.7 Hz, H-5′), 3.43 (s, 3 H, C*H*$_3$O-C6′), 3.40 (ddd, 1 H, $J_{4,5}$ 9.4, $J_{5,6}$ 2.4, 6.6 Hz, H-5); ^{13}C NMR (125 MHz, D_2O) δ 102.1 (C-1, $^1J_{C\text{-}1,H\text{-}1}$ 162.2 Hz, β), 101.4 (C-1′, $^1J_{C\text{-}1',H\text{-}1'}$ 164.4 Hz, β), 79.1 (C-2), 77.3 (C-5), 75.5 (C-5′), 73.5, 73.1 (C-4′, C-4), 72.3 (C-6′), 71.1 (C-2′), 68.2 (C-3′), 67.7 (C-3), 61.8 (C-6), 59.4 (*C*H$_3$O-C6′), 58.0 (*C*H$_3$O-C1); ESI HRMS Calc'd. for $C_{14}H_{26}O_{11}Na$ 393.1367. Found 393.1364.



70

Methyl 4,6-*O*-benzylidene-2-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (70)

Methyl 4,6-*O*-benzylidene-β-D-glucopyranoside[209] **69** (3.0 g, 10.63 mmol), tetrabutylammonium hydrogensulfate (902 mg, 2.66 mmol) and 4-methoxybenzyl chloride (1.8 mL, 13.28 mmol) were dissolved in CH_2Cl_2 (80 mL). Aqueous sodium hydroxide (20 mL of a 5% solution) was added and the mixture was stirred under reflux overnight. The reaction mixture was cooled and the organic layer was separated, washed with water, dried (Na_2SO_4) and then concentrated under reduced pressure. Purification by chromatography on silica gel (4:1, hexanes-EtOAc) gave the 2-*O*-(4-methoxybenzyl) derivative **70** (1.28 g, 30%) and the 3-*O*-(4-methoxybenzyl) derivative (1.93 g, 45%) both as white solids; **70**: R_f 0.48 (1:1, hexanes-EtOAc); $[\alpha]_D$ -18 (*c* 0.73, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48-7.50 (m, 2 H, ArH), 7.30-7.38 (m, 5 H, ArH), 6.89-6.91 (m, 2 H, ArH), 5.53 (s, 1 H, PhC*H*O_2), 4.86 (d, 1 H, J_{gem} 11.1 Hz, PhCH_2O), 4.66 (d, 1 H, PhCH_2O), 4.43 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.35 (dd, 1 H, $J_{5,6}$ 4.9, J_{gem} 10.4 Hz, H-6a), 3.82 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 9.1 Hz, H-3), 3.81 (s, 3 H, CH_3O), 3.78 (dd, 1 H, $J_{5,6} \approx J_{gem}$ 10.3 Hz, H-6b), 3.60 (s, 3 H, CH_3O), 3.53 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.3 Hz, H-4), 3.42 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6}$ 5.1, 9.7 Hz, H-5), 3.32 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 8.9 Hz, H-2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.4 (Ar), 137.0 (Ar), 130.3 (Ar), 129.8 (Ar), 129.2 (Ar), 128.3 (Ar), 126.3 (Ar), 114.0 (Ar), 105.0 (C-1), 101.8 (Ph*C*HO_2), 81.4 (C-2), 80.5 (C-4), 74.3 (PhCH_2O), 73.1 (C-3), 68.7 (C-6), 66.1 (C-5), 57.4 (*C*H_3O), 55.3 (*C*H_3O); ESI HRMS Calc'd. for $C_{22}H_{26}O_7Na$ 425.1571. Found 425.1570.



71

Methyl 4,6-*O*-benzylidene-2-*O*-(4-methoxybenzyl)-3-*O*-thiocarbonylimidazole-β-D-glucopyranoside (71)

The 2-*O*-(4-methoxybenzyl) derivative **70** (1.20 g, 2.98 mmol) was dissolved in dry toluene (20 mL). 1,1'-Thiocarbonyl diimidazole (1.06 g, 5.96 mmol) was added and the reaction was stirred at reflux under argon overnight. The reaction mixture was cooled then concentrated under reduced pressure. The black oily residue was subjected to chromatography on silica gel (7:3, hexanes-EtOAc) to give the Barton-McCombie substrate **71** (978 mg, 64%) as a white solid; R_f 0.24 (1:1, hexanes-EtOAc); $[\alpha]_D$ +10 (*c* 0.84, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 (bs, 1 H, imidazole), 7.44 (bs, 1 H, imidazole), 7.36-7.38 (m, 2 H, ArH), 7.31-7.32 (m, 3 H, ArH), 7.08-7.10 (m, 2 H, ArH), 6.99 (bs, 1 H, imidazole), 6.66-6.67 (m, 2 H, ArH), 6.11 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 9.3 Hz, H-3), 5.46 (s, 1 H, PhC*H*O_2), 4.74 (d, 1 H, J_{gem} 11.8 Hz, PhCH_2O), 4.59 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.54 (d, 1 H, J_{gem} 11.8 Hz, PhCH_2O), 4.41 (dd, 1 H, $J_{5,6}$ 5.0, J_{gem} 10.5 Hz, H-6a), 3.80 (dd, 1 H, $J_{5,6} \approx J_{gem}$ 10.3 Hz, H-6b), 3.76 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4), 3.74 (s, 3 H, CH_3O), 3.64 (s, 3 H, CH_3O), 3.56-3.61 (m, 2 H, H-2, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 183.7 (*C*=S), 159.5 (Ar), 136.7 (Ar), 136.5 (Ar), 130.5 (Ar), 130.0 (Ar), 129.2 (Ar), 129.1 (Ar), 128.2 (Ar), 126.1 (Ar), 118.2 (Ar), 113.6 (Ar), 105.3 (C-1), 101.4 (Ph*C*HO_2), 81.0 (C-3), 78.7 (C-4), 78.0 (C-2/C-5), 73.6 (PhCH$_2$O), 68.7 (C-6), 65.7 (C-2/C-5), 57.7 (*C*H$_3$O), 55.1 (*C*H$_3$O); ESI HRMS Calc'd. for $C_{26}H_{28}N_2O_7SNa$ 535.1509. Found 535.1511; Anal. Calc'd. for $C_{26}H_{28}N_2O_7S$: C,60.92; H, 5.51; N, 5.47; S, 6.26. Found: C, 61.03; H, 5.58; N, 5.46; S, 5.96.

72

Methyl 4,6-*O*-benzylidene-3-deoxy-2-*O*-(4-methoxybenzyl)-β-D-*ribo*-hexopyranoside (72)

To a solution of compound **71** (880 mg, 1.72 mmol) in dry toluene (20 mL) was added tributyltin hydride (1.50 mL, 5.16 mmol) and azobisisobutyronitrile (71 mg, 0.43 mmol). The reaction was stirred at reflux under argon for 16 hours, cooled then concentrated under reduced pressure. The residue was redissolved in a small volume of CH_2Cl_2 and passed through a plug of silica gel containing 10% (w/w) KF. The filtrate was concentrated and the residue subjected to chromatography on silica gel (4:1, hexane-EtOAc) to give the deoxygenated product **72** (515 mg, 78%) as a white solid; R_f 0.55 (1:1, hexanes-EtOAc); $[\alpha]_D$ -40 (*c* 0.76, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47-7.48 (m, 2 H, ArH), 7.34-7.47 (m, 3 H, ArH), 7.26-7.29 (m, 2 H, ArH), 6.87-6.89 (m, 2 H, ArH), 5.49 (s, 1 H, PhC*H*O_2), 4.72 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.59 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.39 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.32 (dd, 1 H, $J_{5,6}$ 5.0, J_{gem} 10.6 Hz, H-6a), 3.81 (s, 3 H, CH_3O), 3.74 (dd, 1 H, $J_{5,6} \approx J_{gem}$ 10.3 Hz, H-6b), 3.59 (s, 3 H, CH_3O), 3.51 (ddd, 1 H, $J_{3eq,4}$ 4.4, $J_{3ax,4}$ 11.9, $J_{4,5}$ 9.1 Hz, H-4), 3.37-3.43 (m, 2 H, H-2, H-5), 2.39 (ddd, 1 H, $J_{2,3} \approx J_{3,4}$ 4.7, J_{gem} 12.1 Hz, H-3eq), 1.74 (ddd, 1 H, $J_{2,3} \approx J_{3,4} \approx J_{gem}$ 11.8 Hz, H-3ax); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.3 (Ar), 137.3 (Ar), 130.4 (Ar), 129.4 (Ar), 129.1 (Ar), 128.3 (Ar), 126.1 (Ar), 113.8 (Ar), 106.4 (C-1), 101.6 (PhCHO$_2$), 76.1 (C-4), 75.1 (C-2), 72.3 (PhCH_2O), 70.0 (C-5), 69.2 (C-6), 57.2 (CH_3O), 55.3

(CH_3O), 34.7 (C-3); ESI HRMS Calc'd. for $C_{22}H_{26}O_6Na$ 409.1622. Found 409.1618; Anal. Calc'd. for $C_{22}H_{26}O_6$: C, 68.38; H, 6.78. Found: C, 68.22; H, 6.77.

73

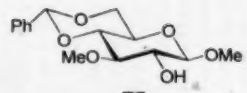
Methyl 4,6-*O*-benzylidene-3-deoxy-β-D-*ribo*-hexopyranoside (73)

To a solution of **72** (499 mg, 1.29 mmol) in CH_2Cl_2 (22 mL) and H_2O (3 mL) at 0 °C (ice-water bath) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (879 mg, 3.87 mmol). The reaction was allowed to slowly warm to room temperature and after 5 hours was diluted with CH_2Cl_2. The organic phase was washed with 10% aqueous $NaHCO_3$, distilled H_2O, brine then dried (Na_2SO_4). Concentration under reduced pressure followed by purification by column chromatography over silica gel (3:2, hexanes-EtOAc) gave **73** (294 mg, 86%) as a white solid; R_f 0.27 (1:1, hexanes-EtOAc); $[\alpha]_D$ -52 (*c* 0.47, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.48-7.50 (m, 2 H, ArH), 7.34-7.40 (m, 3 H, ArH), 5.54 (s, 1 H, PhCHO_2), 4.34 (dd, 1 H, $J_{5,6}$ 4.9, J_{gem} 10.5 Hz, H-6a), 4.26 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.78 (dd, 1 H, $J_{5,6} \approx J_{gem}$ 10.4 Hz, H-6b), 3.57-3.63 (m, 2 H, H-2, H-4), 3.58 (s, 3 H, CH_3O), 3.46 (ddd, 1 H, $J_{4,5}$ 9.1, $J_{5,6}$ 4.9, 10.1 Hz, H-5), 2.47 (ddd, 1 H, $J_{2,3} \approx J_{3,4}$ 4.7, J_{gem} 11.9 Hz, H-3eq), 2.32 (bs, 1 H, OH), 1.76 (ddd, 1 H, $J_{2,3} \approx J_{gem} \approx J_{3,4}$ 11.7 Hz, H-3ax); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.3 (Ar), 129.1 (Ar), 128.3 (Ar), 126.2 (Ar), 106.4 (C-1), 101.8 (PhCHO_2), 76.2 (C-4), 70.6 (C-5), 69.2, 69.1 (C-2, C-6), 57.3 (CH_3O), 35.0 (C-3); ESI HRMS Calc'd. for $C_{14}H_{18}O_5Na$ 289.1047. Found 289.1046; Anal. Calc'd. for $C_{14}H_{18}O_5$: C, 63.15; H, 6.81. Found: C, 63.11; H, 6.80.

74

Methyl 4,6-*O*-benzylidene-3-deoxy-β-D-*arabino*-hexopyranoside (74)

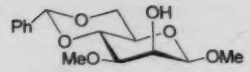
Compound **73** (294 mg, 1.10 mmol) was dissolved in a mixture of freshly distilled Me_2SO (6 mL) and acetic anhydride (3 mL). After stirring for 8 hours at room temperature the reaction was concentrated under reduced pressure. The residue was redissolved in dry THF (10 mL) and cooled to -78 °C. A 1.0 M solution of L-Selectride® in THF (4.4 mL, 4.41 mmol) was added dropwise and stirring was continued for 2 hours at -78 °C. The reaction was quenched with methanol then diluted with CH_2Cl_2. The mixture was washed with 10% aqueous H_2O_2, 1M aqueous NaOH, distilled H_2O then brine. The organic phase was dried (Na_2SO_4) then concentrated under reduced pressure. Purification by chromatography over silica gel (3:2, hexanes-EtOAc) gave **74** (243 mg, 83%) as a white solid; R_f 0.28 (1:1, hexanes-EtOAc); $[\alpha]_D$ -64 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47-7.50 (m, 2 H, ArH), 7.32-7.39 (m, 3 H, ArH), 5.57 (s, 1 H, PhC*H*O_2), 4.50 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1), 4.31 (dd, 1 H, $J_{5,6}$ 5.0, J_{gem} 10.4 Hz, H-6eq), 3.98-4.03 (m, 2 H, H-2, H-4), 3.84 (dd, 1 H, $J_{5,6} \approx J_{gem}$ 10.3, H-6ax), 3.58 (s, 3 H, CH_3O), 3.45 (dd, 1 H, $J_{4,5}$ 9.3, $J_{5,6}$ 5.0, 10.1 Hz, H-5), 2.44 (dd, 1 H, $J_{2,OH} \approx J_{3ax,OH}$ 1.7 Hz, C2-OH), 2.39 (ddd, 1 H, *J* 3.2, 4.5, J_{gem} 13.4 Hz, H-3eq), 1.79 (dddd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 11.8, J_{gem} 13.5, $J_{3,OH}$ 1.9 Hz, H-3ax); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.5 (Ar), 129.0 (Ar), 128.3 (Ar), 126.1 (Ar), 102.0, 101.8 (C-1, PhCHO_2), 73.5 (C-4), 70.6 (C-5), 69.0 (C-6), 67.9 (C-2), 56.8 (CH_3O), 33.9 (C-3); ESI HRMS Calc'd. for $C_{14}H_{18}O_5Na$ 289.1046. Found 289.1043. Anal. Calc'd. for $C_{14}H_{18}O_5$: C, 63.15; H, 6.81. Found: C, 63.05; H, 6.88.



75

Methyl 4,6-*O*-benzylidene-3-*O*-methyl-β-D-glucopyranoside (75)

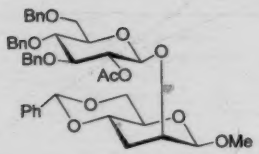
Methyl 4,6-*O*-benzylidene-β-D-glucopyranoside **69** (1.5 g, 5.31 mmol) and dibutyltin oxide (1.46 g, 5.88 mmol) were refluxed overnight in toluene (40 mL) with azeotropic removal of water using a Dean and Stark trap. The reaction mixture was cooled to room temperature then concentrated under reduced pressure. The solid residue was redissolved in DMF (15 mL), methyl iodide (3.3 mL, 53.4 mmol) added and the reaction was stirred at 40 °C. After 15 hours, the reaction was cooled then concentrated under reduced pressure. Purification by chromatography over silica gel (1:1, toluene-EtOAc) gave **75** (1.11 g, 71%) as a white solid; R_f 0.22 (1:1, hexanes-EtOAc); $[\alpha]_D$ -50 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48-7.50 (m, 2 H, ArH), 7.34-7.40 (m, 3 H, ArH), 5.56 (s, 1 H, PhC*H*O_2), 4.36 (dd, 1 H, $J_{5,6}$ 5.0, J_{gem} 10.4 Hz, H-6eq), 4.35 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.80 (dd, 1 H, $J_{5,6} \approx J_{gem}$ 10.3 Hz, H-6ax), 3.68 (s, 3 H, CH_3O), 3.63 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.2 Hz, H-4), 3.59 (s, 3 H, CH_3O), 3.42-3.49 (m, 3 H, H-2, H-3, H-5), 2.53 (d, 1 H, $J_{2,OH}$ 1.9 Hz, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.2 (Ar), 129.0 (Ar), 128.3 (Ar), 126.0 (Ar), 104.2 (C-1), 101.3 (PhCHO_2), 82.2 (C-3), 81.6 (C-4), 74.1 (C-2), 68.7 (C-6), 66.4 (C-5), 60.9 (CH_3O), 57.4 (CH_3O); ESI HRMS Calc'd. for $C_{15}H_{20}O_6Na$ 319.1152. Found 319.1152; Anal. Calc'd. for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80;. Found: C, 60.92; H, 6.75.



76

Methyl 4,6-*O*-benzylidene-3-*O*-methyl-β-D-mannopyranoside (76)

Compound **75** (446 mg, 1.51 mmol) was dissolved in a mixture of Me_2SO (6 mL) and acetic anhydride (3 mL). After stirring overnight the mixture was concentrated under reduced pressure. The remaining solid was redissolved in CH_2Cl_2 (5 mL) and methanol (5 mL) and cooled to 0 °C (ice-water bath). Sodium borohydride (286 mg, 7.53 mmol) was added and the reaction was stirred until starting material was consumed according to TLC. The reaction was diluted with CH_2Cl_2 then washed successively with 2% aqueous citric acid, brine, dried (Na_2SO_4) then concentrated under reduced pressure. Chromatography over silica gel (7:3, hexanes-EtOAc) gave **76** (356 mg, 80%) as a white solid; R_f 0.07 (1:1, hexanes-EtOAc); $[\alpha]_D$ -74 (*c* 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.48-7.50 (m, 2 H, ArH), 7.33-7.38 (m, 3 H, ArH), 5.59 (s, 1 H, PhC*H*O_2), 4.48 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 4.35 (dd, 1 H, $J_{5,6}$ 5.0, J_{gem} 10.3 Hz, H-6eq), 4.21 (d, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 4.06 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4), 3.89 (dd, 1 H, $J_{5,6} \approx J_{gem}$ 10.3 Hz, H-6ax), 3.59 (s, 3 H, C*H*$_3$O), 3.58 (s, 3 H, C*H*$_3$O), 3.47 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.6 Hz, H-3), 3.39 (ddd, 1 H, $J_{4,5}$ 9.8, $J_{5,6}$ 4.9, 9.8 Hz, H-5), 2.49 (bs, 1 H, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.4 (Ar), 129.0 (Ar), 128.2 (Ar), 126.1 (Ar), 101.7, 101.4 (C-1, PhCHO$_2$), 79.3 (C-3), 78.4 (C-4), 69.0 (C-2), 68.6 (C-6), 66.8 (C-5), 58.6 (*C*H$_3$O), 57.3 (*C*H$_3$O); ESI HRMS Calc'd. for $C_{15}H_{20}O_6Na$ 319.1152. Found 319.1154. Anal. Calc'd. for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80. Found: C, 61.20; H, 6.73.

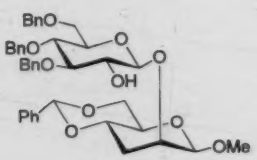


77

Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl (1→2) 4,6-*O*-benzylidene-3-deoxy-β-D-*arabino*-hexopyranoside (77)

Monosaccharide acceptor **74** (120 mg, 0.45 mmol) was reacted with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate **21** (344 mg, 0.54 mmol) in CH_2Cl_2 (5 mL) using TMSOTf (5 μL, 0.03 mmol) under argon at 0 °C then processed as described for **22**. The product was purified by chromatography over silica gel (7:3, hexanes-EtOAc) to give **77** (196 mg, 59%) as a white solid; R_f 0.52 (1:1, hexanes-EtOAc); $[\alpha]_D$ -24 (*c* 0.24, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46-7.48 (m, 2 H, ArH), 7.25-7.38 (m, 16 H, ArH), 7.18-7.20 (m, 2 H, ArH), 5.57 (s, 1 H, PhC*H*O_2), 5.00 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.3 Hz, H-2'), 4.81 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.78 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.73 (d, 1 H, J_{gem} 11.2 Hz, PhCH_2O), 4.73 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.62 (d, 1 H, J_{gem} 12.4 Hz, PhCH_2O), 4.57 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O); 4.55 (d, 1 H, J_{gem} 12.3 Hz, PhCH_2O), 4.39 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 4.26 (dd, 1 H, $J_{5,6}$ 4.9, J_{gem} 10.3 Hz, H-6eq), 4.03 (ddd, 1 H, $J_{1,2}$ 0.9, $J_{2,3}$ 3.1 Hz, H-2), 3.93 (ddd, 1 H, $J_{3,4}$ 4.6, 12.6 Hz, $J_{4,5}$ 9.5 Hz, H-4), 3.80 (dd, 1 H, $J_{5,6} \approx J_{gem}$ 10.2 Hz, H-6ax), 3.63-3.74 (m, 4 H, H-3', H-4', H-6a', H-6b'), 3.48 (ddd, 1 H, $J_{4',5'}$ 9.6, $J_{5',6'}$ 2.6, 4.7 Hz, H-5'), 3.47 (s, 3 H, CH_3O), 3.40 (ddd, 1 H, $J_{4,5}$ 9.4, $J_{5,6}$ 4.9, 10.1 Hz, H-5), 2.39 (ddd, 1 H, $J_{2,3}$ 4.0, J_{gem} 12.7, $J_{3,4}$ 4.0 Hz, H-3eq), 2.00 (s, 3 H, C*H*$_3$C(O)O), 1.76 (ddd, 1 H, $J_{2,3}$ 2.7, J_{gem} 12,4, $J_{3,4}$ 12.4 Hz, H-3ax); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.9 (C=O), 138.4 (Ar), 138.2 (Ar), 138.0 (Ar),

137.7 (Ar), 128.9 (Ar), 128.3(8) (Ar), 128.3(6) (Ar), 128.3 (Ar), 128.0 (Ar), 127.8 (Ar), 127.7(4) (Ar), 127.6(7) (Ar), 127.6(2) (Ar), 127.5(7) (Ar), 126.1 (Ar), 103.2 (C-1), 101.8 (PhCHO$_2$), 101.5 (C-1′), 82.8 (C-3′), 77.9 (C-4′), 75.0(4), 75.0(0), 74.8 (C-5′, PhCH$_2$O×2), 73.7, 73.6 (C-2′, C-2, C-4), 73.4 (PhCH$_2$O), 71.1 (C-5), 69.0, 68.9 (C-6′, C-6), 56.8 (CH$_3$O), 34.7 (C-3), 21.1 (CH$_3$C(O)O); ESI HRMS Calc'd. for $C_{43}H_{48}O_{11}Na$ 763.3089. Found 763.3084; Anal. Calc'd. for $C_{43}H_{48}O_{11}$: C, 69.71; H, 6.53. Found: C, 69.68; H, 6.64.

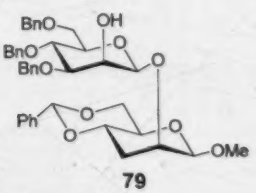


78

Methyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl (1→2) 4,6-*O*-benzylidene-3-deoxy-β-D-*arabino*-hexopyranoside (78)

Disaccharide **77** (176 mg, 0.24 mmol) was dissolved in CH_2Cl_2-methanol (1:1, 2 mL) and treated with 0.5 M MeONa/MeOH (2 mL) then processed as described for **23**. Purification of the product by chromatography over silica gel (7:3, hexanes-EtOAc) gave **78** (166 mg, quant.) as a white solid; R_f 0.33 (1:1, hexanes-EtOAc); $[\alpha]_D$ -5 (*c* 0.82, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47-7.50 (m, 2 H, ArH), 7.23-7.42 (m, 16 H, ArH), 7.18-7.20 (m, 2 H, Ar), 5.59 (s, 1 H, PhC*H*O$_2$), 5.05 (d, 1 H, J_{gem} 11.2 Hz, PhC*H*$_2$O), 4.87 (d, 1 H, J_{gem} 10.9 Hz, PhC*H*$_2$O), 4.80 (d, 1 H, J_{gem} 11.2 Hz, PhC*H*$_2$O), 4.60 (d, 1 H, J_{gem} 12.2 Hz, PhC*H*$_2$O), 4.55 (d, 1 H, J_{gem} 12.4 Hz, PhC*H*$_2$O), 4.54 (d, 1 H, J_{gem} 10.8 Hz, PhC*H*$_2$O), 4.50 (s, 1 H, H-1), 4.41 (d, 1 H, $J_{1',2'}$ 7.3 Hz, H-1′), 4.32 (dd, 1 H,

$J_{5,6}$ 4.9, J_{gem} 10.4 Hz, H-6eq), 4.01-4.06 (m, 2 H, H-2, H-4), 3.87 (dd, 1 H, $J_{5,6} \approx J_{gem}$ 10.4 Hz, H-6ax), 3.63-3.74 (m, 4 H, H-2′, H-3′, H-6a′, H-6b′), 3.56-3.60 (m, 4 H, H-4′, CH_3O), 3.44-3.52 (m, 2 H, H-5′, H-5), 2.55 (ddd, 1 H, $J_{2,3} \approx J_{3,4}$ 3.8, J_{gem} 13.1 Hz, H-3eq), 1.84 (ddd, 1 H, $J_{2,3}$ 1.8, $J_{3,4} \approx J_{gem}$ 12.9 Hz, H-3ax); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.9 (Ar), 138.2 (Ar), 138.1 (Ar), 137.5 (Ar), 129.0 (Ar), 128.3(8) (Ar), 128.3(5) (Ar), 128.3(2) (Ar), 128.3(1) (Ar), 128.0(0) (Ar), 127.9(8) (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 126.1 (Ar), 106.0 (C-1′), 102.5 (C-1), 101.9 (PhCHO$_2$), 84.8 (C-2′), 77.6 (C-2), 77 (C-4′), 75.6, 75.3 (C-5′, C-3), 75.1 (PhCH$_2$O), 74.9 (PhCH$_2$O), 73.7, 73.5 (C-4, PhCH$_2$O), 70.9 (C-5), 69.0(2), 68.9(7) (C-6′, C-6), 57.2 (CH$_3$O), 35.0 (C-3); ESI HRMS Calc'd. for $C_{41}H_{46}O_{10}Na$ 721.2983. Found 721.2986. Anal. Calc'd. for $C_{41}H_{46}O_{10}$: C, 70.47; H, 6.63. Found: C, 70.47; H, 6.68.

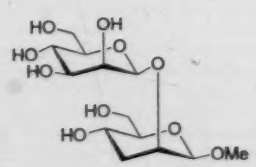


79

Methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl (1→2) 4,6-*O*-benzylidene-3-deoxy-β-D-arabino-hexopyranoside (79)

Disaccharide **78** (140 mg, 0.200 mmol) was dissolved in freshly distilled Me_2SO (5 mL) and acetic anhydride (5 mL) then processed as described for compound **24**. The concentrated reaction mixture was then treated with 1.0 M L-Selectride® in THF (1 mL, 1 mmol) in dry THF (10 mL) at -78 °C under argon. Purification by column chromatography over silica gel (7:3, hexanes-EtOAc) gave **79** (90.8 mg, 65%) as a white

solid; R_f 0.26 (1:1, hexanes-EtOAc); $[\alpha]_D$ -15 (*c* 0.60, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.47-7.49 (m, 2 H, ArH), 7.21-7.39 (m, 18 H, ArH), 5.56 (s, 1 H, PhC*H*O_2), 4.91 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.80 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.71 (d, 1 H, $J_{1',2'}$ 0.8 Hz, H-1′), 4.65 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.60 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O), 4.56 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.55 (d, 1 H, J_{gem} 12.3 Hz, PhCH_2O), 4.46 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 4.31 (dd, 1 H, $J_{5,6}$ 4.9, J_{gem} 10.4 Hz, H-6eq), 4.29 (dd, 1 H, $J_{1',2'}$ 0.9, $J_{2',3'}$ 3.0 Hz, H-2′), 4.14 (ddd, 1 H, H-2), 4.01 (ddd, 1 H, $J_{3,4}$ 3.3, 12.0, $J_{4,5}$ 9.2 Hz, H-4), 3.87 (dd, 1 H, $J_{5,6} \approx J_{gem}$ 10.3 Hz, H-6ax), 3.85 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.3 Hz, H-4′), 3.76 (dd, 1 H, $J_{5',6'}$ 2.0, J_{gem} 10.8 Hz, H-6a′), 3.68 (dd, 1 H, $J_{5',6'}$ 5.7, J_{gem} 10.8 Hz, H-6b′), 3.57 (dd, 1 H, $J_{2',3'}$ 3.0, $J_{3',4'}$ 9.1 Hz, H-3′), 3.52 (s, 3 H, CH_3O), 3.42-3.48 (m, 2 H, H-5′, H-5), 2.51 (ddd, 1 H, $J_{2,3} \approx J_{3,4}$ 4.0, J_{gem} 13.1 Hz, H-3eq), 1.83 (ddd, 1 H, $J_{2,3}$ 2.9, J_{gem} 13.0, $J_{3,4}$ 12.0 Hz, H-3ax); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.2(8), 138.2(6), 137.9, 137.5, 129.0, 128.4, 128.3(4), 128.2(8), 128.1, 127.9, 127.8, 127.7(1), 127.6(9), 127.5, 126.1, 103.2, 101.8, 100.9, 81.4, 75.4, 75.1, 74.3, 74.1, 73.8, 73.5, 71.1(3), 71.0(9), 69.5, 68.9, 67.8, 57.3, 34.7; ESI HRMS Calc'd. for $C_{41}H_{46}O_{10}Na$ 721.2983. Found 721.2985.

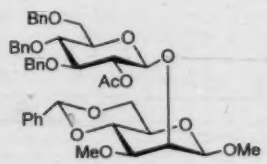


12

Methyl β-D-mannopyranosyl (1→2) 3-deoxy-β-D-*arabino*-hexopyranoside (12)

Compound **79** (49.0 mg, 0.070 mmol) was dissolved in CH_2Cl_2 (5 mL)-methanol (5 mL) and stirred with 10% Pd/C (50 mg) under an H_2 atmosphere then processed as

described for **1**. Filtration then lyophilization gave **12** (17.8 mg, 75%) as a clear glass; R_f 0.25 (6:3.5:0.5, CH_2Cl_2-MeOH-H_2O); $[\alpha]_D$ -33 (*c* 0.27, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 4.78 (d, 1 H, $J_{1',2'}$ 0.8 Hz, H-1′), 4.63 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 4.14 (m, 1 H, H-2), 4.05 (dd, 1 H, $J_{1',2'}$ 0.8, $J_{2',3'}$ 3.3 Hz, H-2′), 3.92 (dd, 1 H, $J_{5',6'}$ 2.3, J_{gem} 12.3 Hz, H-6a′), 3.91 (dd, 1 H, $J_{5,6}$ 2.7, J_{gem} 12.2 Hz, H-6a), 3.83 (ddd, 1 H, $J_{3,4}$ 4.7, 11.0, $J_{4,5}$ 9.6 Hz, H-4), 3.73 (dd, 1 H, $J_{5',6'}$ 6.5, J_{gem} 12.3 Hz, H-6b′), 3.72 (dd, 1 H, $J_{5,6}$ 6.8, J_{gem} 12.2 Hz, H-6b), 3.63 (dd, 1 H, $J_{2',3'}$ 3.3, $J_{3',4'}$ 9.6 Hz, H-3′), 3.56 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.6 Hz, H-4′), 3.53 (s, 3 H, CH_3O), 3.50 (ddd, 1 H, $J_{4,5}$ 9.3, $J_{5,6}$ 2.7, 6.8 Hz, H-5), 3.35 (ddd, 1 H, $J_{4',5'}$ 9.6, $J_{5',6'}$ 2.3, 6.5 Hz, H-5′), 2.41 (ddd, 1 H, $J_{2,3} \approx J_{3,4}$ 4.3, J_{gem} 13.6 Hz, H-3eq), 1.72 (ddd, 1 H, $J_{2,3}$ 2.9, J_{gem} 13.6, $J_{3,4}$ 11.1 Hz, H-3ax); ^{13}C NMR (125 MHz, D_2O) δ 102.9 (C-1, $^1J_{C\text{-}1,H\text{-}1}$ 160.3 Hz, β), 101.8 (C-1′, $^1J_{C\text{-}1',H\text{-}1'}$ 160.6 Hz, β), 80.9 (C-5), 77.1 (C-5′), 75.7 (C-2), 73.7 (C-3′), 71.3 (C-2′), 67.7 (C-4′), 63.1 (C-4), 62.2, 62.0 (C-6′, C-6), 57.6 (*C*H_3O), 36.9 (C-3); ESI HRMS Calc'd. for $C_{13}H_{24}O_{10}Na$ 363.1262. Found 363.1264.

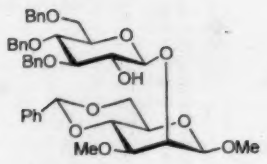


80

Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl (1→2) 4,6-*O*-benzylidene-3-*O*-methyl-β-D-mannopyranoside (80)

Monosaccharide acceptor **76** (205 mg, 0.69 mmol) was reacted with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate **21** (484 mg, 0.76 mmol) in CH_2Cl_2 (5 mL) using TMSOTf (6 μL, 0.03 mmol) under argon at 0 °C then processed as

described for **22**. The product was purified by chromatography over silica gel (7:3, hexanes-EtOAc) to give **80** (513 mg, 97%) as a white solid; R_f 0.49 (1:1, hexanes-EtOAc); $[\alpha]_D$ -40 (*c* 0.67, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46-8.48 (m, 2 H, ArH), 7.27-7.36 (m, 16 H, ArH), 7.21-7.22 (m, 2 H, ArH), 5.57 (s, 1 H, PhC*H*O_2), 5.10 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.6 Hz, H-2′), 4.82 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.79 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.77 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.75 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.58 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.57 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.54 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.35 (s, 1 H, H-1), 4.28 (dd, 1 H, $J_{5,6}$ 4.7, J_{gem} 10.1 Hz, H-6eq), 4.26 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.99 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.6 Hz, H-4), 3.82 (dd, 1 H, $J_{5,6} \approx J_{gem}$ 10.2 Hz, H-6b), 3.75 (dd, 1 H, $J_{2',3'}$ 9.5, $J_{3',4'}$ 8.5 Hz, H-3′), 3.74 (dd, 1 H, $J_{5',6'}$ 1.8, J_{gem} 10.9 Hz, H-6a′), 3.63 (dd, 1 H, $J_{5',6'}$ 6.2, J_{gem} 10.9 Hz, H-6b′), 3.57 (dd, 1 H, $J_{3',4'}$ 8.6, $J_{4',5'}$ 9.8 Hz, H-4′), 3.53 (ddd, 1 H, $J_{4',5'}$ 9.8, $J_{5',6'}$ 1.7, 6.0 Hz, H-5′), 3.49 (s, 3 H, C*H*$_3$O), 3.46 (s, 3 H, C*H*$_3$O), 3.37 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 10.0 Hz H-3), 3.33 (ddd, 1 H, $J_{4,5}$ 9.9, $J_{5,6}$ 5.0, 9.9 Hz, H-5), 1.99 (s, 3 H, C*H*$_3$C(O)O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.7 (C=O), 138.5 (Ar), 138.2 (Ar), 138.0 (Ar), 137.5 (Ar), 128.9 (Ar), 128.4 (Ar), 128.3(4) (Ar), 128.3(3) (Ar), 128.2 (Ar), 128.1 (Ar), 127.8 (Ar), 127.7(2) (Ar), 127.6(9) (Ar), 127.5(9) (Ar), 127.5(7) (Ar), 126.2 (Ar), 102.7 (C-1), 101.9 (PhC*H*O_2), 101.1 (C-1′), 83.0 (C-3′), 78.6 (C-3), 78.1 (C-4′), 77.7 (C-4), 75.1, 75.0, 74.8 (C-5′, PhCH_2O×2), 73.6(1), 73.5(8), 73.2 (C-2′, C-2, PhCH_2O), 69.7 (C-6′), 68.6 (C-6), 67.7 (C-5), 57.0 (*C*H$_3$O), 56.8 (*C*H$_3$O), 21.1 (*C*H$_3$C(O)O); ESI HRMS Calc'd. for $C_{44}H_{50}O_{12}Na$ 793.3195. Found 793.3195.

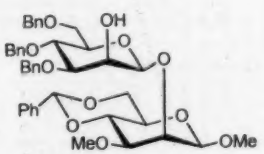


81

Methyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl (1→2) 4,6-*O*-benzylidene-3-*O*-methyl-β-D-mannopyranoside (81)

Disaccharide **80** (513 mg, 0.67 mmol) was dissolved in CH_2Cl_2-methanol (1:1, 4 mL) and treated with 0.5 M MeONa/MeOH (1 mL) then processed as described for **23**. Purification of the product by chromatography over silica gel (7:3, hexanes-EtOAc) gave **81** (428 mg, 88%) as a white solid; R_f 0.27 (1:1, hexanes-EtOAc); $[\alpha]_D$ -38 (*c* 0.37, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47-7.49 (m, 2 H, ArH), 7.42-7.46 (m, 16 H, ArH), 7.19-7.21 (m, 2 H, ArH), 5.55 (s, 1 H, PhC*H*O_2), 5.06 (d, 1 H, J_{gem} 11.2 Hz, PhCH_2O), 4.87 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.81 (d, 1 H, J_{gem} 11.2 Hz, PhCH_2O), 4.54 (s, 2 H, PhCH_2O), 4.54 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.50 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.46 (d, 1 H, $J_{1,2}$ 0.6 Hz, H-1), 4.34 (dd, 1 H, $J_{5,6}$ 4.9, J_{gem} 10.4 Hz, H-6eq), 4.26 (d, 1 H, $J_{2,3}$ 3.2, H-2), 4.02 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.7 Hz, H-4), 3.87 (dd, 1 H, $J_{5,6} \approx J_{gem}$ 10.3 Hz, H-6ax), 3.76 (dd, 1 H, $J_{5',6'}$ 1.7, J_{gem} 10.5 Hz, H-6a′), 3.74 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 8.9 Hz, H-2′), 3.67 (dd, 1 H, $J_{2',3'} \approx J_{3',4'}$ 8.8 Hz, H-3′), 3.63 (dd, 1 H, $J_{5',6'}$ 6.4, J_{gem} 10.5 Hz, H-6b′), 3.58 (s, 3 H, C*H*$_3$O), 3.56 (ddd, 1 H, $J_{4',5'}$ 10.0, $J_{5',6'}$ 1.7, 6.5 Hz, H-5′), 3.47 (dd, 1 H, $J_{3',4'}$ 8.5, $J_{4',5'}$ 9.7 Hz, H-4′), 3.48 (s, 3 H, C*H*$_3$O), 3.42 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 10.0 Hz, H-3), 3.38 (ddd, 1 H, $J_{4,5}$ 9.9, $J_{5,6}$ 4.9, 9.9 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.0 (Ar), 138.3 (Ar), 138.2 (Ar), 137.4 (Ar), 128.9 (Ar), 128.4 (Ar), 128.3(1) (Ar), 128.2(9) (Ar), 128.2 (Ar), 128.1 (Ar), 128.0 (Ar), 127.8 (Ar), 127.7 (Ar), 127.5(4) (Ar), 127.4(8)

(Ar), 126.2 (Ar), 105.1 (C-1′), 102.1 (C-1), 101.8 (PhCHO$_2$), 85.2 (C-3′), 78.8 (C-3), 77.8 (C-4), 77.3 (C-4′), 76.4 (C-2), 75.4(9), 75.4(6) (C-2′, C-5′), 75.0 (PhCH$_2$O), 74.8 (PhCH$_2$O), 73.5 (PhCH$_2$O), 69.8 (C-6′), 68.6 (C-6), 67.5 (C-5), 57.5 (CH$_3$O), 57.4 (CH$_3$O); ESI HRMS Calc'd. for $C_{42}H_{48}O_{11}Na$ 751.3089. Found 751.3089. Anal. Calc'd. for $C_{42}H_{48}O_{11}$: C, 69.21; H, 6.64. Found: C, 68.95; H, 6.82.

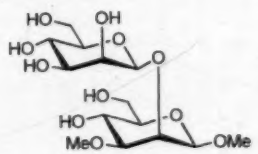


82

Methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl (1→2) 4,6-*O*-benzylidene-3-*O*-methyl-β-D-mannopyranoside (82)

Disaccharide **81** (428 mg, 0.59 mmol) was dissolved in freshly distilled Me_2SO (10 mL) and acetic anhydride (5 mL) then processed as described for compound **24**. The concentrated reaction mixture was then treated with 1.0 M L-Selectride® in THF (2.4 mL, 2.35 mmol) in dry THF (10 mL) at -78 °C under argon. Purification by column chromatography over silica gel (7:3, hexanes-EtOAc) gave **82** (308 mg, 72%) as a white solid; R_f 0.19 (1:1, hexanes-EtOAc); $[\alpha]_D$ -60 (*c* 0.47, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49-7.50 (m, 2 H, ArH), 7.24-7.40 (m, 18 H, ArH), 5.58 (s, 1 H, PhC*H*O$_2$), 4.94 (d, 1 H, J_{gem} 11.0 Hz, PhC*H*$_2$O), 4.85 (d, 1 H, $J_{1',2'}$ 0.8 Hz, H-1′), 4.82 (d, 1 H, J_{gem} 12.1 Hz, PhC*H*$_2$O), 4.65 (d, 1 H, J_{gem} 12.0 Hz, PhC*H*$_2$O), 4.58 (d, 1 H, J_{gem} 12.0 Hz, PhC*H*$_2$O), 4.57 (d, 1 H, J_{gem} 11.0 Hz, PhC*H*$_2$O), 4.52 (m, 1 H, H-2), 4.51 (d, 1 H, J_{gem} 12.0 Hz, PhC*H*$_2$O), 4.42 (d, 1 H, $J_{1,2}$ 0.9 Hz, H-1), 4.33 (dd, 1 H, $J_{5,6}$ 5.0, J_{gem} 10.4 Hz, H-6eq), 4.28 (dd, 1 H, $J_{1',2'}$ 0.7, $J_{2',3'}$ 3.0 Hz, H-2′), 4.01 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.6 Hz, H-4),

3.89 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.4 Hz, H-4′), 3.88 (dd, 1 H, $J_{5,6}$ 10.3 Hz, H-6ax), 3.77 (dd, 1 H, $J_{5',6'}$ 2.0, J_{gem} 10.8 Hz, H-6a′), 3.71 (dd, 1 H, $J_{5',6'}$ 5.8 J_{gem} 10.8 Hz, H-6b′), 3.55 (dd, 1 H, $J_{2',3'}$ 3.0, $J_{3',4'}$ 9.1 Hz, H-3′), 3.52 (s, 3 H, CH_3O), 3.47 (ddd, 1 H, $J_{4',5'}$ 9.7, $J_{5',6'}$ 1.9, 5.7 Hz, H-5′), 3.45 (s, 3 H, CH_3O), 3.43 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 9.9 Hz, H-3), 3.38 (ddd, 1 H, $J_{4,5}$ 9.9, $J_{5,6}$ 4.9, 9.9 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4 (Ar), 138.3 (Ar), 138.1 (Ar), 137.3 (Ar), 129.0 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.0 (Ar), 127.9 (Ar), 127.7 (Ar), 127.6(4) (Ar), 127.5(7) (Ar), 126.2 (Ar), 102.9 (C-1), 101.8 (Ph*C*HO$_2$), 98.1 (C-1′), 81.4 (C-3′), 78.8 (C-3), 77.7 (C-4), 75.3, 75.1, 74.3 (C-4′, C-5′, Ph*C*H$_2$O), 73.4 (PhCH$_2$O), 70.8, 70.7 (C-2, PhCH$_2$O), 69.8 (C-6′), 68.6 (C-6), 67.8, 67.5 (C-2′, C-5), 57.5 (*C*H$_3$O), 57.1 (*C*H$_3$O); ESI HRMS Calc'd. for $C_{42}H_{48}O_{11}Na$ 751.3089. Found 751.3087; Anal. Calc'd. for $C_{42}H_{48}O_{11}$: C, 69.21; H, 6.64. Found: C, 68.85; H, 6.70.

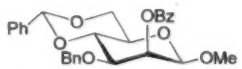


13

Methyl β-D-mannopyranosyl (1→2) 3-*O*-methyl-β-D-mannopyranoside (13)

Compound **82** (50.7 mg, 0.070 mmol) was dissolved in CH_2Cl_2 (5 mL)-methanol (5 mL) and stirred with 10% Pd/C (50 mg) under an H_2 atmosphere then processed as described for **1**. Filtration then lyophilization gave **13** (19.1 mg, 74%) as a clear glass; R_f 0.23 (6.0:3.5:0.5, CH_2Cl_2-methanol-H_2O); $[\alpha]_D$ -86 (*c* 0.37, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 4.83 (s, 1 H, H-1′), 4.60 (s, 1 H, H-1), 4.52 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 4.09 (d, 1 H, $J_{2',3'}$ 1.8 Hz, H-2′), 3.93 (dd, 1 H, $J_{5,6}$ 2.0, J_{gem} 12.3 Hz, H-6a), 3.93 (dd, 1 H, $J_{5',6'}$ 1.8, J_{gem}

12.2 Hz, H-6a′), 3.77 (dd, 1 H, $J_{5',6'}$ 5.8, J_{gem} 12.2 Hz, H-6b′), 3.74 (dd, 1 H, $J_{5,6}$ 6.5, J_{gem} 12.3 Hz, H-6b), 3.64 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.9 Hz, H-4), 3.58-3.64 (m, 2 H, H-3′, H-4′), 3.54 (s, 3 H, CH_3O), 3.49 (s, 3 H, CH_3O), 3.41 (ddd, 1 H, $J_{4,5}$ 9.2, $J_{5,6}$ 2.2, 6.5 Hz, H-5), 3.38 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.8 Hz, H-3), 3.34 (ddd, 1 H, $J_{4',5'}$ 9.5, $J_{5',6'}$ 2.2, 5.8 Hz, H-5′); ^{13}C NMR (125 MHz, D_2O) δ 102.6 (C-1, $^1J_{C\text{-}1,H\text{-}1}$ 158.5 Hz, β), 101.4 (C-1′, $^1J_{C\text{-}1',H\text{-}1'}$ 164.1 Hz, β), 82.2 (C-3), 77.3, 77.0 (C-5, C-5′), 73.9, 73.6 (C-2, C-4′), 71.3 (C-2′), 67.5, 66.7 (C-4, C-3′), 62.0, 61.9 (C-6, C-6′), 58.0 (*C*H$_3$O-C1), 57.2 (*C*H$_3$O-C3); ESI HRMS Calc'd. for $C_{14}H_{26}O_{11}Na$ 393.1367. Found 393.1366.

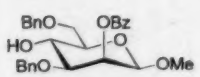


84

Methyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (84)

Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside[209] **83** (1.54 g, 4.14 mmol) was dissolved in pyridine (40 mL). Benzoyl chloride (1.5 mL, 12.41 mmol) was added and the reaction was stirred at room temperature overnight. The reaction mixture was then concentrated under reduced pressure. The residue was dissolved in EtOAc then washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, distilled H_2O and brine. The organic phase was dried (Na_2SO_4) then concentrated under reduced pressure. Column chromatography (4:1, hexanes-EtOAc) on silica gel gave **84** (1.97 g, quant.) as a white solid; R_f 0.51 (1:1, hexanes-EtOAc); $[\alpha]_D$ -115 (*c* 0.26, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13-8.16 (m, 2 H, ArH), 7.23-7.59 (m, 13 H, ArH), 5.88 (dd, 1 H, $J_{1,2}$ 1.1, $J_{2,3}$ 3.4 Hz, H-2), 5.68 (s, 1 H, PhC*H*O$_2$), 4.79 (d, 1 H, J_{gem} 12.6 Hz, PhCH_2O), 4.69 (d, 1 H,

PhCH_2O), 4.60 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 4.41 (dd, 1 H, $J_{5,6eq}$ 4.9, J_{gem} 10.5 Hz, H-6eq), 4.14 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.6 Hz, H-4), 3.98 (dd, 1 H, $J_{5,6ax}$ 9.7, J_{gem} 10.4 Hz, H-6ax) 3.84 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 9.8 Hz, H-3), 3.53 (s, 3H, CH_3O), 3.47 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6}$ 4.8, 9.7 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1 (*C*=O), 137.7 (Ar), 137.4 (Ar), 133.1 (Ar), 130.1 (Ar), 129.9 (Ar), 129.0 (Ar), 128.3(3) (Ar), 128.3(0) (Ar), 128.2 (Ar), 127.7 (Ar), 126.1 (Ar), 101.6 (PhCHO$_2$), 101.1 (C-1), 78.4 (C-4), 75.6 (C-3), 71.6 (PhCH$_2$O), 69.3 (C-2), 68.7 (C-6), 67.4 (C-5), 57.5 (CH$_3$O); ESI HRMS Calc'd. for $C_{28}H_{28}O_7Na$ 499.1727. Found 499.1729. Anal. Calc'd. for $C_{28}H_{28}O_7$: C, 70.57; H, 5.92. Found: C, 70.81; H, 5.73.

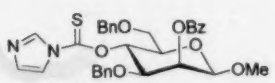


85

Methyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-mannopyranoside (85)

Compound **84** (3.43 g, 6.77 mmol) was dissolved in dry THF and the solution cooled to 0 °C (ice-water bath) under argon. Activated 4 Å molecular sieves and $NaCNBH_3$ (2.13 g, 33.86 mmol) were added. A saturated solution of HCl in diethyl ether was added dropwise until the solution was acidic (pH paper, gas evolution). After 3 h, the reaction was diluted with EtOAc and filtered through celite. The organic phase was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by column chromatography (4:1, hexanes-EtOAc) to give **85** (2.80 g, 81%) as a white solid; R_f 0.40 (1:1, hexanes-EtOAc); $[\alpha]_D$ -113 (*c* 0.22, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07-8.09 (m, 2 H, ArH), 7.54 (m, 1 H, ArH), 7.26-7.41 (m, 12 H, ArH), 5.84 (dd, 1 H, $J_{1,2}$ 0.9, $J_{2,3}$ 3.1 Hz, H-2) 4.85 (d, 1 H,

J_{gem} 11.4 Hz, PhCH_2O) 4.72 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O) 4.64 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O) 4.55 (d, 1 H, $J_{1,2}$ 0.9 Hz, H-1) 4.50 (d, 1 H, J_{gem} 11.4 Hz, PhCH_2O), 4.04 (ddd, 1 H, $J_{4,OH}$ 2.0, $J_{3,4} \approx J_{4,5}$ 9.4 Hz, H-4), 3.92 (ABX, 1 H, $J_{5,6}$ 3.2, J_{gem} 10.7 Hz, H-6a), 3.88 (ABX, 1 H, $J_{5,6}$ 5.3, J_{gem} 10.7 Hz, H-6b), 3.54-3.58 (m, 2 H, H-3, H-5), 3.53 (s, 3 H, CH_3O), 2.57 (d, 1 H, $J_{4,OH}$ 2.1 Hz, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1 (*C*=O), 138.2 (Ar), 137.3 (Ar), 133.0 (Ar), 130.1 (Ar), 129.9 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.0 (Ar), 127.6 (Ar), 100.3 (C-1), 79.6 (C-3), 75.3 (C-5), 73.7 (Ph*C*H$_2$O), 71.2 (Ph*C*H$_2$O), 70.2 (C-6), 67.8(4), 67.8(0) (C-2, C-4), 57.2 (*C*H$_3$O); ESI HRMS Calc'd. for $C_{28}H_{30}O_7Na$ 501.1884. Found 501.1884.

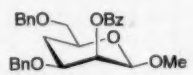


86

Methyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-thiocarbonyl imidazole-β-D-mannopyranoside (86)

To a solution of **85** (1.43 g, 2.98 mmol) in dry toluene (20 mL) under argon was added 1,1′-thiocarbonyl diimidazole (1.60 g, 8.95 mmol). The reaction was stirred at 90 °C. After 20 h, the reaction was cooled to room temperature then concentrated under reduced pressure to give a brown oily residue. Purification of the product by chromatography (1:1, hexanes-EtOAc) yielded **86** (1.05 g, 60%) as a white solid; R_f 0.28 (1:1, hexanes-EtOAc); $[\alpha]_D$ -166 (*c* 0.29, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13-8.15 (m, 2 H, ArH), 8.10 (m, 1 H, ArH), 7.59 (m, 1 H, ArH), 7.43-7.46 (m, 3 H, ArH), 7.12-7.28 (m, 10 H, ArH), 7.02 (m, 1 H, ArH), 6.24 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.3 Hz, H-4), 5.90

(dd, 1 H, $J_{1,2}$ 0.9, $J_{2,3}$ 3.2 Hz, H-2), 4.69 (d, 1 H, J_{gem} 12.8 Hz, PhCH_2O), 4.61 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 4.54 (AB, 1 H, J_{gem} 11.7 Hz, PhCH_2O), 4.52 (AB, 1 H, J_{gem} 11.7 Hz, PhCH_2O), 4.45 (d, 1 H, J_{gem} 12.8 Hz, PhCH_2O), 3.83 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.4 Hz, H-3), 3.72-3.82 (m, 3 H, H-5, H-6a, H-6b), 3.45 (s, 3 H, CH_3O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 183.4 (*C*=S), 166.0 (*C*=O), 137.6 (Ar), 136.8 (Ar), 136.6 (Ar), 133.2 (Ar), 130.8 (Ar), 130.2 (Ar), 129.7 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 127.9 (Ar), 127.7 (Ar), 127.6 (Ar), 118.2 (Ar), 100.2 (C-1), 77.2 (C-4), 75.8 (C-3), 73.9 (Ph*C*H_2O), 73.6 (C-5), 70.6 (Ph*C*H_2O), 69.7 (C-6), 67.8 (C-2), 57.4 (*C*H_3O); ESI HRMS Calc'd. for $C_{32}H_{32}N_2O_7SNa$ 611.1822. Found 611.1820; Anal. Calc'd. for $C_{32}H_{32}N_2O_7S$: C, 65.29; H, 5.48; N, 4.76; S, 5.45. Found: C, 65.55; H, 5.53; N, 4.76; S, 5.27.

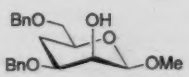


87

Methyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-deoxy-β-D-*lyxo*-hexopyranoside (87)

To a solution of the Barton-McCombie substrate **86** (1.05 g, 1.78 mmol) in dry toluene (15 mL) was added tributyltin hydride (1.43 mL, 5.33 mmol) and azobisisobutyronitrile (73 mg, 0.25 mmol). The reaction was refluxed under argon overnight then cooled to room temperature. The reaction mixture was filtered through a plug of silica gel containing 10% (w/w) KF. The filtrate was concentrated then subjected to chromatography (4:1, hexanes-EtOAc) to give **87** (562.2 mg, 68%) as a white solid; R_f 0.55 (1:1, hexanes-EtOAc); $[\alpha]_D$ -101 (*c* 0.59, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11-8.12 (m, 2 H, ArH), 7.53-7.56 (m, 1 H, ArH), 7.25-7.43 (m, 12 H, ArH), 5.78 (dd, 1 H, $J_{1,2}$ 0.8, $J_{2,3}$ 2.8 Hz, H-2), 4.74 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.65 (s, 2 H, PhCH_2O),

4.52 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.42 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 3.76 (dd, 1 H, $J_{5,6}$ 5.5, J_{gem} 9.4 Hz, H-6a), 3.68-3.74 (m, 2 H, H-3, H-5), 3.65 (dd, 1 H, $J_{5,6}$ 4.2, J_{gem} 9.4 Hz, H-6b) 3.51 (s, 3 H, CH_3O), 1.91-1.94 (m, 2 H, H-4a, H-4b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.2 (*C*=O), 138.3 (Ar), 137.8 (Ar), 132.9 (Ar), 130.2 (Ar), 130.1 (Ar), 128.4 (Ar), 128.2 (Ar), 127.7(1) (Ar), 127.6(8) (Ar), 100.7 (C-1), 74.0 (C-3), 73.6 (PhCH_2O), 72.7 (C-6), 72.0 (C-5), 70.0 (PhCH_2O), 67.2 (C-2), 57.1 (CH_3O), 30.0 (C-4); ESI HRMS Calc'd. for $C_{28}H_{30}O_6Na$ 485.1935. Found 485.1936.

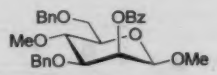


88

Methyl 3,6-di-*O*-benzyl-4-deoxy-β-D-*lyxo*-hexopyranoside (88)

Compound **87** (534.3 mg, 1.16 mmol) was dissolved in methanol (10 mL). A solution of 0.5 M MeONa/MeOH (6 mL) was added then the reaction stirred at room temperature until TLC (1:1, hexanes:EtOAc) indicated the consumption of starting material. The reaction was neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered and the filtrate concentrated under reduced pressure. Purification by chromatography (1:1, hexanes-EtOAc) gave **88** (369.5 mg, 89%) as a white solid; R_f 0.30 (1:1, hexanes-EtOAc); $[\alpha]_D$ -45 (*c* 1.15, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28-7.36 (m, 10 H, ArH), 4.56-4.66 (m, 4 H, PhCH_2O×4), 4.25 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 4.04 (bs, 1 H, H-2), 3.67 (m, 1 H, H-6a), 3.51-3.60 (m, 3 H, H-3, H-5, H-6b), 3.56 (s, 3 H, CH_3O), 2.28 (d, 1 H, $J_{2,OH}$ 2.4 Hz, OH), 1.84 (m, 1 H, H-4a), 1.76 (m, 1 H, H-4b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.2 (Ar), 137.9 (Ar), 128.5 (Ar), 128.4 (Ar), 127.8 (Ar), 127.7(2) (Ar), 127.6(9) (Ar), 127.6(7) (Ar), 101.3 (C-1), 75.0 (C-3), 73.6 (PhCH_2O), 72.7 (C-6), 71.7

(C-5), 69.9 (PhCH$_2$O), 67.3 (C-2), 56.9 (CH$_3$O), 28.3 (C-4); ESI HRMS Calc'd. for $C_{21}H_{26}O_5Na$ 381.1672. Found 381.1671.

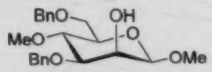


89

Methyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-methyl-β-D-mannopyranoside (89)

Compound **85** (643.3 mg, 1.34 mmol) was dissolved in dry DMF (10 mL) under argon. Methyl iodide (167 μL, 2.69 mmol) was added and the reaction cooled to 0 °C (ice-water bath). Sodium hydride (107.5 mg, 2.69 mmol; 60% in oil) was added in one portion and the reaction stirred at 0 °C for 3 h. The reaction was quenched with acetic acid then concentrated under reduced pressure. Purification of the product by chromatography (4:1, hexanes-EtOAc) yielded **89** (643.8 mg, 97%) as a white solid; R_f 0.54 (1:1, hexanes-EtOAc); $[\alpha]_D$ -96 (*c* 0.22, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09-8.11 (m, 2 H, ArH), 7.53 (m, 1 H, ArH), 7.42-7.44 (m, 2 H, ArH), 7.26-7.38 (m, 10 H, ArH), 5.81 (dd, 1 H, $J_{1,2}$ 0.8, $J_{2,3}$ 3.0 Hz, H-2), 4.83 (d, 1 H, J_{gem} 11.6 Hz, PhCH_2O), 4.81 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.65 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.58 (d, 1 H, J_{gem} 11.6 Hz, PhCH_2O), 4.49 (d, 1 H, $J_{1,2}$ 0.9 Hz, H-1), 3.90 (dd, 1 H, $J_{5,6}$ 4.3, J_{gem} 11.0 Hz, H-6a), 3.87 (dd, 1 H, $J_{5,6}$ 2.1, J_{gem} 11.0 Hz, H-6b), 3.71 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.3 Hz, H-4), 3.66 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.2 Hz, H-3), 3.55 (s, 3 H, CH_3O), 3.52 (s, 3 H, CH_3O), 3.46 (ddd, 1 H, $J_{4,5}$ 9.3, $J_{5,6}$ 2.1, 4.3 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.0 (*C*=O), 138.6 (Ar), 137.8 (Ar), 132.9 (Ar), 130.1 (Ar), 128.3(4) (Ar), 128.3(3) (Ar), 128.2(6) (Ar), 128.0 (Ar), 127.7 (Ar), 127.5(0) (Ar), 127.4(6) (Ar), 100.2 (C-1), 80.1 (C-

3), 76.2 (C-4), 75.8 (C-5), 73.5 (Ph*C*H$_2$O), 71.2 (Ph*C*H$_2$O), 69.3 (C-6), 68.4 (C-2), 61.0 (*C*H$_3$O), 57.2 (*C*H$_3$O); ESI HRMS Calc'd. for $C_{29}H_{32}O_7Na$ 515.2040. Found 515.2042; Anal. Calc'd. for $C_{29}H_{32}O_7$: C, 70.71; H, 6.55. Found: C, 70.58; H, 6.54.

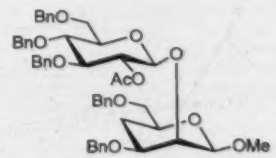


90

Methyl 3,6-di-*O*-benzyl-4-*O*-methyl-β-D-mannopyranoside (90)

To a solution of mannopyranoside **89** (793.3 mg, 1.61 mmol) in CH_2Cl_2 (10 mL) and methanol (10 mL) was added 0.5 M MeONa/MeOH (6 mL) and the reaction stirred overnight. The reaction was neutralized with Amberlite resin (IR-120 H^+), the resin was filtered and the filtrate concentrated under reduced pressure. Purification of the product by chromatography (1:1, hexanes-EtOAc) gave **90** (592.5 mg, 95%) as a white solid; R_f 0.28 (1:1, hexanes-EtOAc); $[\alpha]_D$ -34 (*c* 0.44, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26-7.41 (m, 10 H, ArH), 4.76 (d, 1 H, J_{gem} 11.9 Hz, PhC*H*$_2$O), 4.69 (d, 1 H, J_{gem} 11.8 Hz, PhC*H*$_2$O), 4.67 (d, 1 H, J_{gem} 12.0 Hz, PhC*H*$_2$O), 4.60 (d, 1 H, J_{gem} 12.1 Hz, PhC*H*$_2$O), 4.31 (d, 1 H, H-1), 4.06 (m, 1 H, H-2), 3.80 (dd, 1 H, $J_{5,6}$ 2.2, J_{gem} 10.8 Hz, H-6a), 3.75 (dd, 1 H, $J_{5,6}$ 5.3, J_{gem} 10.9 Hz, H-6b), 3.57 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.3 Hz, H-4), 3.55 (s, 3 H, C*H*$_3$O), 3.53 (s, 3 H, C*H*$_3$O), 3.46 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.0 Hz, H-3), 3.36 (ddd, 1 H, $J_{4,5}$ 9.6, $J_{5,6}$ 2.1, 5.3 Hz, H-5), 2.39 (d, 1 H, $J_{2,OH}$ 2.4 Hz, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4 (Ar), 138.0 (Ar), 128.5 (Ar), 128.3 (Ar), 127.8(2) (Ar), 127.8(0) (Ar), 127.7 (Ar), 127.5 (Ar), 100.7 (C-1), 81.3 (C-3), 76.2 (C-4), 75.4 (C-5), 73.6

(PhCH_2O), 71.5 (PhCH_2O), 69.4 (C-6), 68.3 (C-2), 60.8 (CH_3O), 56.9 (CH_3O); ESI HRMS Calc'd. for $C_{22}H_{28}O_6Na$ 411.1778. Found 411.1778.

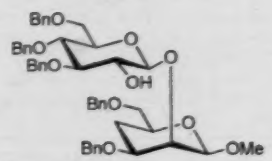


91

Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl (1→2) 3,6-di-*O*-benzyl-4-deoxy-β-D-*lyxo*-hexopyranoside (91)

Monosaccharide acceptor **88** (347 mg, 0.97 mmol) was reacted with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate **21** (758 mg, 1.19 mmol) in CH_2Cl_2 (5 mL) using TMSOTf (5 μL, 0.03 mmol) under argon at 0 °C (ice-water bath) then processed as described for **22**. The product was purified by chromatography over silica gel (4:1, hexanes-EtOAc) to give **91** (669 mg, 83%) as a clear syrup; R_f 0.59 (1:1, hexanes-EtOAc); $[\alpha]_D$ -55 (*c* 0.43, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18-7.34 (m, 25 H, ArH), 5.09 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.5 Hz, H-2′), 4.84 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′) 4.81 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.80 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O), 4.78 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.74 (d, 1 H, J_{gem} 11.4 Hz, PhCH_2O), 4.54-4.59 (m, 2 H, PhCH_2O), 4.54 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.47 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O), 4.42-4.47 (m, 2 H, PhCH_2O), 4.17 (d, 1 H, $J_{2,3}$ 2.5 Hz, H-2), 4.11 (s, 1 H, H-1), 3.72-3.77 (m, 2 H, H-3′, H-6a′), 3.54-3.63 (m, 5 H, H-4′, H-5′, H-6b′ H-5, H-6a), 3.48 (dd, 1 H, $J_{5,6}$ 4.0, J_{gem} 9.7 Hz, H-6b), 3.46 (s, 3 H, CH_3O), 3.40 (ddd, 1 H, $J_{2,3}$ 2.7, $J_{3,4eq}$ 4.7, $J_{3,4ax}$ 11.7 Hz, H-3), 1.96 (s, 3 H, CH_3C(O)O), 1.75 (ddd, 1 H, $J_{3,4} \approx J_{gem} \approx J_{4,5}$ 12.0 Hz, H-4ax),

1.65 (ddd, 1 H, $J_{3,4}$ 4.5, J_{gem} 12.6, $J_{4,5}$ 2.2 Hz, H-4eq); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.7 (*C*=O), 138.5 (Ar), 138.4 (Ar), 138.3 (Ar), 138.2 (Ar), 138.0 (Ar), 128.4 (Ar), 128.3(2) (Ar), 128.2(9) (Ar), 128.2(6) (Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7(2) (Ar), 127.6(7) (Ar), 127.6(1) (Ar), 127.5(8) (Ar), 127.5(5) (Ar), 127.4(9) (Ar), 127.4 (Ar), 102.4 (C-1), 100.9 (C-1′), 83.1 (C-3′), 78.2 (C-4′/C-5′/C-5), 75.0, 74.9, 74.7 (C-4′/C-5′/C-5, Ph*C*H_2O×2), 73.9 (Ph*C*H_2O), 73.7 (C-3), 73.5, 73.4, 73.2 (C-2′, C-6, Ph*C*H_2O), 72.0 (C-4′/C-5′/C-5), 71.4 (C-2), 69.9 (C-6′), 68.7 (Ph*C*H_2O), 56.5 (*C*H_3O), 29.2 (C-4), 21.1 (*C*H_3C(O)O); ESI HRMS Calc'd. for $C_{50}H_{56}O_{11}Na$ 855.3715. Found 855.3710; Anal. Calc'd. for $C_{50}H_{56}O_{11}$: C, 72.10; H, 6.78. Found: C, 72.10; H, 6.79.

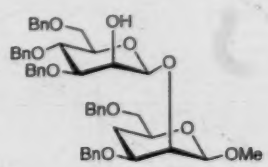


92

Methyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl (1→2) 3,6-di-*O*-benzyl-4-deoxy-β-D-*lyxo*-hexopyranoside (92)

Disaccharide **91** (448 mg, 0.54 mmol) was dissolved in CH_2Cl_2-methanol (1:1, 10 mL) and treated with 0.5 M MeONa/MeOH (2 mL) then processed as described for **23**. Purification of the product by chromatography over silica gel (7:3, hexanes-EtOAc) gave **92** (334 mg, 78%) as a colourless syrup; R_f 0.42 (1:1, hexanes-EtOAc); $[\alpha]_D$ -60 (*c* 0.88, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40-7.42 (m, 2 H, ArH), 7.22-7.37 (m, 21 H, ArH), 7.17-7.18 (m, 2 H, ArH), 5.06 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.86 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.79 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.78 (d, 1 H, J_{gem} 12.5 Hz,

PhCH_2O), 4.61 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O) 4.59 (d, 1 H, $J_{1',2'}$ 8.8 Hz, H-1'), 4.56 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.52 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.51 (d, 1 H, J_{gem} 12.5 Hz, PhCH_2O), 4.46 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.42 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.22 (s, 1 H, H-1), 4.15 (d, 1 H, $J_{2,3}$ 2.5 Hz, H-2) 3.44-3.74 (m, 11 H, H-2', H-3', H-4', H-5', H-6a', H-6b', OH, H-3, H-5, H-6a, H-6b), 3.54 (s, 3 H, CH_3O), 1.85 (ddd, 1 H, $J_{3,4} \approx J_{gem} \approx J_{4,5}$ 12.5 Hz, H-4ax), 1.78 (ddd, 1 H, J 2.0, 4.6, J_{gem} 12.4 Hz, H-4eq); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.1 (Ar), 138.3 (Ar), 138.2(3) (Ar), 138.1(8) (Ar), 128.4 (Ar), 128.3(1) (Ar), 128.2(5) (Ar), 128.0 (Ar), 127.9 (Ar), 127.7(3) (Ar), 127.6(9) (Ar), 127.6(8) (Ar), 127.6(5) (Ar), 127.5 (Ar), 127.4(4) (Ar), 127.3(9) (Ar), 104.5 (C-1'), 101.9 (C-1), 85.2, 77.4, 75.5, 75.4, 75.0, 74.7, 74.3, 74.1, 73.6, 73.4, 72.7, 72.1, 69.7, 69.3 (C-2', C-3', C-4', C-5', C-6', C-2, C-3, C-5, C-6, PhCH_2O×5), 56.9 (CH_3O), 29.4 (C-4); ESI HRMS Calc'd. for $C_{48}H_{54}O_{10}Na$ 813.3609. Found 813.3608.

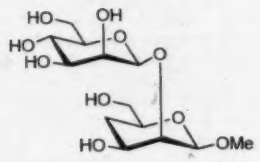


93

Methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl (1→2) 3,6-di-*O*-benzyl-4-deoxy-β-D-*lyxo*-hexopyranoside (93)

Disaccharide **92** (312 mg, 0.39 mmol) was dissolved in Me_2SO (5 mL), acetic anhydride (2.5 mL) was added and the reaction stirred overnight. The reaction mixture was concentrated under reduced pressure. The residue was redissolved in dry THF (5 mL) and the solution cooled to -78 °C. L-Selectride® (1.7 mL, 1.0 M in THF) was added

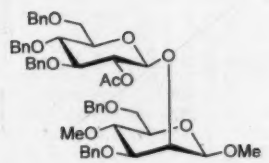
dropwise and the reaction was allowed to slowly warm to room temperature. The reaction was quenched with methanol and concentrated under reduced pressure. Purification by column chromatography over silica gel (7:3, hexanes-EtOAc) gave **93** (200 mg, 65%) as a white solid; R_f 0.28 (1:1, hexanes-EtOAc); $[\alpha]_D$ -66 (*c* 0.73, $CHCl_3$) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21-7.41 (m, 25 H, ArH), 4.95 (d, 1 H, J_{gem} 11.1 Hz, PhCH_2O), 4.93 (d, 1 H, $J_{1',2'}$ 0.7 Hz, H-1′), 4.87 (d, 1 H, J_{gem} 11.4 Hz, PhCH_2O), 4.83 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O), 4.64 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.60 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.55 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.54 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.45 (d, 1 H, $J_{2,3}$ 2.7 Hz, H-2), 4.41 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.38-4.43 (m, 2 H, PhCH_2O), 4.33 (d, 1 H, $J_{2',3'}$ 2.8 Hz, H-2′), 4.21 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1), 3.88 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.3 Hz, H-4′), 3.75 (dd, 1 H, $J_{5',6'}$ 1.8, J_{gem} 10.5 Hz, H-6a′), 3.66 (dd, 1 H, $J_{5,6}$ 5.9, J_{gem} 9.6 Hz, H-6a), 3.47-3.62 (m, 6 H, H-3′, H-5′, H-6b′, H-3, H-5, H-6b), 3.50 (s, 3 H, CH_3O), 2.74 (bs, 1 H, OH), 1.74-1.85 (m, 2 H, H-4ax, H-4eq); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5 (Ar), 138.2(2) (Ar), 138.2(1) (Ar), 138.1(7) (Ar), 138.1(5) (Ar), 128.3(9) (Ar), 128.3(6) (Ar), 128.3(5) (Ar), 128.3(0) (Ar), 128.2(7) (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.7(0) (Ar), 127.6(8) (Ar), 127.6(4) (Ar), 127.5(9) (Ar), 127.5(8) (Ar), 127.5(3) (Ar), 127.4(7) (Ar), 102.7 (C-1), 98.8 (C-1′), 81.4 (C-3′), 75.0(9), 75.0(5), 74.5, 74.0, 73.6, 73.3, 72.8 (C-3, C-6, C-4′, C-5′, PhCH_2O×3), 72.0 (C-5), 70.7 (PhCH_2O), 70.0 (C-6′), 69.2 (PhCH_2O), 68.7 (C-2), 67.7 (C-2′), 57.1 (CH_3O), 29.7 (C-4); ESI HRMS Calc'd. for $C_{48}H_{54}O_{10}Na$ 813.3609. Found 813.3609.



14

Methyl β-D-mannopyranosyl (1→2) 4-deoxy-β-D-*lyxo*-hexopyranoside (14)

Compound **93** (49.1 mg, 0.062 mmol) was dissolved in CH_2Cl_2 (5 mL)-methanol (5 mL) and stirred with 10% Pd/C (50 mg) under an H_2 atmosphere then processed as described for **1**. Filtration then lyophilization gave **14** (11.0 mg, 52%) as a clear glass; R_f 0.27 (6:3.5:0.5, CH_2Cl_2-MeOH-H_2O); $[\alpha]_D$ -60 (*c* 0.58, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 4.83 (s, 1 H, H-1′), 4.51 (s, 1 H, H-1), 4.12-4.13 (m, 2 H, H-2′, H-2), 3.92 (dd, 1 H, $J_{5',6'}$ 2.3, J_{gem} 12.3 Hz, H-6a′), 3.88 (ddd, 1 H, $J_{2,3}$ 3.0, $J_{3,4eq}$ 5.0, $J_{3,4ax}$ 12.1 Hz, H-3) 3.63-3.73 (m, 5 H, H-3′, H-6b′, H-5, H-6a, H-6b) 3.55 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.8, H-4′), 3.54 (s, 3 H, CH_3O), 3.36 (ddd, 1 H, $J_{5',6b'}$ 6.8 Hz, H-5′), 1.71 (ddd, 1 H, J_{gem} 12.6, $J_{4,5}$ 0.8 Hz, H-4eq), 1.55 (ddd, 1 H, $J_{4ax,5}$ 12.2 Hz, H-4ax); ^{13}C NMR (125 MHz, D_2O) δ 102.7 (C-1, $^1J_{C-1,H-1}$ 159.4 Hz, β), 101.3 (C-1′, $^1J_{C-1',H-1'}$ 162.4 Hz, β), 77.4 (C-2), 77.2 (C-5′), 74.0, 73.7 (C-3′, C-5), 71.3 (C-2′), 68.3 (C-3), 67.7 (C-4′), 64.6 (C-6), 62.0 (C-6′), 57.8 (CH_3O), 30.7 (C-4); ESI HRMS Calc'd. for $C_{13}H_{24}O_{10}Na$ 363.1262. Found 363.1261.

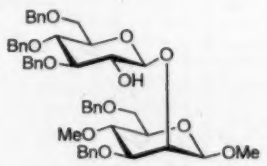


94

Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl (1→2) 3,6-di-*O*-benzyl-4-*O*-methyl-β-D-mannopyranoside (94)

Monosaccharide acceptor **90** (238 mg, 0.44 mmol) was reacted with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate **21** (469 mg, 0.74 mmol) in CH_2Cl_2 (5 mL) using TMSOTf (7 μL, 0.04 mmol) under argon at 0 °C then processed as described for **22**. The product was purified by chromatography over silica gel (4:1, hexanes-EtOAc) to give **94** (511 mg, 97%) as a colourless syrup; R_f 0.54 (1:1, hexanes-EtOAc); $[\alpha]_D$ -53 (*c* 0.65, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17-7.39 (m, 25 H, ArH), 5.09 (dd, 1 H, $J_{1',2'}$ 8.1, $J_{2',3'}$ 9.6 Hz, H-2'), 4.86 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.80 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.79 (d, 1 H, H-1'), 4.78 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.74 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.61 (d, 1 H, J_{gem} 12.3 Hz, PhCH_2O), 4.57 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O), 4.54 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.52 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.43-4.48 (m, 2 H, PhCH_2O), 4.18-4.19 (m, 2 H, H-1, H-2), 3.71-3.79 (m, 3 H, H-3', H-6a', H-6a), 3.56-3.66 (m, 4 H, H-4', H-5', H-6b', H-6b), 3.51 (s, 3 H, CH_3O), 3.47 (s, 3 H, CH_3O), 3.36 (dd, 1 H, $J_{2,3}$ 2.9, $J_{3,4}$ 8.9 Hz, H-3), 3.34 (ddd, 1 H, $J_{4,5}$ 9.5 Hz, $J_{5,6}$ 1.6, 6.8 Hz, H-5), 3.28 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.2 Hz, H-4), 1.95 (s, 3 H, CH_3C(O)O)); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8 (*C*=O), 138.6 (Ar), 138.5 (Ar), 138.3 (Ar), 138.1 (Ar), 138.0 (Ar), 128.4 (Ar), 128.3(2) (Ar), 128.3(0) (Ar), 128.2 (Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.5(7) (Ar), 127.5(6) (Ar), 127.4(5) (Ar),

127.4 (Ar), 101.6, 101.1 (C-1, C-1′), 83.1 (C-3′), 79.9 (C-3), 78.0, 76.4, 75.7, 74.9, 74.8, 74.7, 73.5(4), 73.4(8), 73.3, 72.4, 70.7, 69.9, 69.7 (C-2′, C-4′, C-5′, C-6′, C-2, C-4, C-5, C-6, PhCH_2O×5), 61.0 (CH_3O), 56.6 (CH_3O), 21.0 (CH_3C(O)O); ESI HRMS Calc'd. for $C_{51}H_{58}O_{12}Na$ 885.3821. Found 885.3828.

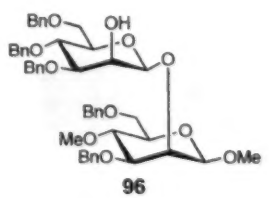


95

Methyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl (1→2) 3,6-di-*O*-benzyl-4-*O*-methyl-β-D-mannopyranoside (95)

Disaccharide **94** (511 mg, 0.59 mmol) was dissolved in CH_2Cl_2-methanol (1:1, 10 mL) and treated with 0.5 M MeONa/MeOH (5 mL) then processed as described for **23**. Purification of the product by chromatography over silica gel (7:3, hexanes-EtOAc) gave **95** (470 mg, 97%) as a clear syrup; R_f 0.38 (1:1, hexanes-EtOAc); $[\alpha]_D$ -52 (*c* 0.20, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24-7.41 (m, 23 H, ArH), 7.16-7.18 (m, 2 H, ArH), 5.06 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.84-4.87 (m, 2 H, PhCH_2O), 4.78 (d, 1 H, J_{gem} 11.2 Hz, PhCH_2O), 4.67 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.61 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1′), 4.59 (d, 1 H, J_{gem} 11.1 Hz, PhCH_2O), 4.56 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.52 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.47 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.44 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.27 (s, 1 H, H-1), 4.19 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 3.78 (dd, 1 H, $J_{5,6}$ 2.1, J_{gem} 10.9 Hz, H-6a), 3.73 (dd, 1 H, $J_{5,6}$ 5.1, J_{gem} 11.0 Hz, H-6b), 3.49-3.72 (m, 7 H, H-2′, H-3′, H-4′, H-5′, H-6a′, H-6b′, H-4), 3.52 (s, 6 H, CH_3O×2), 3.41 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$

9.3 Hz, H-3), 3.32 (ddd, 1 H, $J_{4,5}$ 9.5, $J_{5,6}$ 2.0 ,4.9 Hz, H-5), 3.31 (bs, 1 H, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.1 (Ar), 138.4 (Ar), 138.2(5) (Ar), 138.1(8) (Ar), 138.1(7) (Ar), 128.3(1) (Ar), 128.2(8) (Ar), 128.2 (Ar), 128.0 (Ar), 127.9(4) (Ar), 127.9(0) (Ar), 127.7 (Ar), 127.6(4) (Ar), 127.6(3) (Ar), 127.5 (Ar), 127.4 (Ar), 104.2 (C-1′), 101.5 (C-1), 85.2 (C-3′), 80.1 (C-3), 77.1 (C-5′), 76.0, 75.8 (C-4, C-5), 75.4, 75.3, 75.0, 74.8, 74.7 (C-2, C-2′, C-4′, $PhCH_2O$×2), 73.5 ($PhCH_2O$), 73.4 ($PhCH_2O$), 70.4 ($PhCH_2O$), 69.7 (C-6′), 69.4 (C-6), 61.0 (CH_3O), 57.1 (CH_3O); ESI HRMS Calc'd. for $C_{49}H_{56}O_{11}Na$ 843.3715. Found 843.3719. Anal. Calc'd. for $C_{49}H_{56}O_{11}$: C, 71.69; H, 6.88. Found: C, 71.48; H, 7.09.

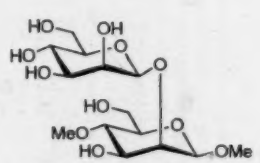


96

Methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl (1→2) 3,6-di-*O*-benzyl-4-*O*-methyl-β-D-mannopyranoside (96)

As described for compound **24**, disaccharide **95** (446 mg, 0.54 mmol) was dissolved in freshly distilled Me_2SO (10 mL) and acetic anhydride (5 mL). The concentrated reaction mixture was then treated with 1.0 M L-Selectride® in THF (2.2 mL, 2.17 mmol) in dry THF (10 mL) at -78 °C under argon. Purification by column chromatography over silica gel (7:3, hexanes-EtOAc) gave **96** (273 mg, 61%) as a white solid; R_f 0.29 (1:1, hexanes-EtOAc); $[\alpha]_D$ -60 (*c* 0.47, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.21-740 (m, 25 H, ArH), 4.94 (d, 1 H, J_{gem} 10.9 Hz, $PhCH_2O$), 4.89-4.90 (m, 2 H, H-1′, $PhCH_2O$), 4.82 (d, 1 H, J_{gem} 12.1 Hz, $PhCH_2O$), 4.66 (d, 1 H, J_{gem} 12.1 Hz,

PhCH_2O), 4.63 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.57 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.54 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.41-4.48 (m, 4 H, H-2, 3(PhCH_2O)), 4.31 (dd, 1 H, $J_{1',2'}$ 0.7, $J_{2',3'}$ 3.0 Hz, H-2'), 4.26 (d, 1 H, $J_{1,2}$ 0.6 Hz, H-1), 3.88 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.5 Hz, H-4'), 3.78 (dd, 1 H, $J_{5,6}$ 2.1, J_{gem} 10.9 Hz, H-6a), 3.75 (dd, 1 H, $J_{5',6'}$ 2.0, J_{gem} 10.4 Hz, H-6a'), 3.73 (dd, 1 H, $J_{5,6}$ 5.4, J_{gem} 10.8 Hz, H-6b), 3.62 (dd, 1 H, $J_{5',6'}$ 6.2, J_{gem} 10.6 Hz, H-6b'), 3.56 (dd, 1 H, $J_{2',3'}$ 3.0, $J_{3',4'}$ 9.1 Hz, H-3'), 3.47-3.52 (m, 2 H, H-5', H-4), 3.49 (s, 3 H, CH_3O), 3.48 (s, 3 H, CH_3O), 3.43 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 9.2 Hz, H-3), 3.33 (ddd, 1 H, $J_{4,5}$ 9.6, $J_{5,6}$ 1.9, 5.4 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4(4) (Ar), 138.3(6) (Ar), 138.3 (Ar), 138.2 (Ar), 138.1 (Ar), 128.4 (Ar), 128.3(1) (Ar), 128.2(6) (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5(4) (Ar), 127.5(3) (Ar), 127.4 (Ar), 102.1 (C-1), 99.1 (C-1'), 81.5 (C-3'), 80.1 (C-3), 75.9, 75.7, 75.1, 75.0, 74.4 (C-4, C-5, C-4', C-5', PhCH_2O), 73.5 (PhCH_2O), 73.4 (PhCH_2O), 70.7 (PhCH_2O), 70.3 (C-2), 69.9(7), 69.9(6), 69.6 (C-6, C-6', PhCH_2O), 67.7 (C-2'), 60.9 (CH_3O), 57.2 (CH_3O); ESI HRMS Calc'd. for $C_{49}H_{56}O_{11}Na$ 843.3715. Found 843.3716.

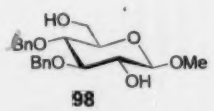


15

Methyl β-D-mannopyranosyl (1→2) 4-*O*-methyl-β-D-mannopyranoside (15)

Compound **96** (51.8 mg, 0.063 mmol) was dissolved in CH_2Cl_2 (5 mL)-methanol (5 mL) and stirred with 10% Pd/C (50 mg) under an H_2 atmosphere then processed as described for **1**. Filtration then lyophilization gave **15** (20.4 mg, 88%) as a clear glass; R_f

0.48 (6:3.5:0.5, CH_2Cl_2-methanol-H_2O); $[\alpha]_D$ -56 (*c* 0.50, H_2O); ^1H NMR (500 MHz, D_2O) δ 4.82 (s, 1 H, H-1′), 4.61 (s, 1 H, H-1), 4.23 (d, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 4.12 (d, 1 H, $J_{2',3'}$ 3.3 Hz, H-2′), 3.91-3.93 (m, 2 H, H-6a′, H-6a), 3.71-3.77 (m, 3 H, H-6b′, H-3, H-6b), 3.63 (ddd, 1 H, $J_{2',3'}$ 0.9, $J_{3',4'}$ 9.7 Hz, H-3′), 3.56 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.7 Hz, H-4′), 3.53 (s, 6 H, C*H*$_3$OC-1, C*H*$_3$OC-4), 3.34-3.39 (m, 3 H, H-5′, H-4, H-5); ^{13}C NMR (125 MHz, D_2O) δ 102.2 (C-1, $^1J_{C\text{-}1,H\text{-}1}$ 159.7 Hz, β), 101.3 (C-1′, $^1J_{C\text{-}1',H\text{-}1'}$ 162.7 Hz, β), 78.7 (C-2), 78.0 (C-4), 77.2 (C-5′), 76.3 (C-5), 73.7 (C-3′), 73.0 (C-3), 71.2 (C-2′), 67.7 (C-4′), 62.0, 61.5 (C-6′, C-6), 61.1 (*C*H$_3$OC-4), 58.0 (*C*H$_3$OC-1); ESI HRMS Calc'd. for $C_{14}H_{26}O_{11}Na$ 393.1367. Found 393.1366.

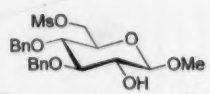


98

Methyl 3,4-di-*O*-benzyl-β-D-glucopyranoside (98)

Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside[209] **97** (1.56 g, 4.19 mmol) in a flame dried flask was dissolved in dry CH_2Cl_2 (20 mL) and cooled to 0 °C (ice-water bath) under argon. A 1.0 M solution of BH_3·THF complex in THF (21 mL, 20.95 mmol) was added followed by the dropwise addition of a 1.0 M solution of dibutylboron triflate (2.1 mL, 2.10 mmol). After 3 hours, the reaction was quenched with methanol, neutralized with Et_3N then concentrated under reduced pressure. Chromatography over silica gel (1:1, hexanes-EtOAc) yielded **98** (1.30 g, 83%) as a white solid; R_f 0.12 (1:1, hexanes-EtOAc); $[\alpha]_D$ -10 (*c* 0.25, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.28-7.40 (m, 10 H, ArH), 4.93 (d, 1 H, J_{gem} 11.3 Hz, PhC*H*$_2$O), 4.89 (d, 1 H,

J_{gem} 11.0 Hz, PhCH_2O), 4.88 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.67 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.24 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.89 (ddd, 1 H, $J_{5,6}$ 2.7, J_{gem} 12.0, $J_{6,OH}$ 5.5 Hz, H-6a), 3.75 (ddd, 1 H, $J_{5,6}$ 4.4, J_{gem} 12.0, $J_{6,OH}$ 8.0 Hz, H-6b), 3.60-3.62 (m, 2 H, H-3, H-4), 3.56 (s, 3 H, CH_3O), 3.50 (m, 1 H, H-2), 3.40 (ddd, 1 H, $J_{4,5}$ 9.2, $J_{5,6}$ 2.8, 4.4 Hz, H-5), 2.45 (d, 1 H, $J_{2,OH}$ 2.3 Hz, C2-OH), 1.97 (dd, 1 H, $J_{6,OH}$ 5.7, 8.0 Hz, C6-OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5 (Ar), 137.9 (Ar), 128.5 (Ar), 128.1 (Ar), 127.9(4) (Ar), 127.9(3) (Ar), 127.8(Ar), 103.8 (C-1), 84.3, 77.3 (C-3, C-4), 75.4, 75.2, 75.1 (C-5, Ph*C*H_2O×2), 74.6 (C-2), 61.9 (C-6), 57.3 (*C*H_3O); ESI HRMS Calc'd. for $C_{21}H_{26}O_6Na$ 397.1622. Found 397.1619; Anal. Calc'd. for $C_{21}H_{26}O_6$: C, 67.36; H, 7.00. Found: C, 67.10; H, 7.02.

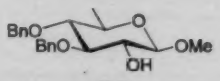


99

Methyl 3,4-di-*O*-benzyl-6-*O*-methanesulfonyl-β-D-glucopyranoside (99)

Compound **98** (489 mg, 1.31 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and dry pyridine (5 mL) and the mixture was cooled to 0 °C (ice-water bath) under argon. Methanesulfonyl chloride (152 μL, 1.96 mmol) was added dropwise and the reaction was stirred overnight. The reaction mixture was diluted with CH_2Cl_2 and washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, distilled water, brine then dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography over silica gel (1:1, toluene-EtOAc) yielded **99** (389 mg, 66%) as a white solid; R_f 0.23 (1:1, hexanes-EtOAc); $[\alpha]_D$ -17 (*c* 0.34 $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29-7.40 (m, 10 H, ArH), 4.96 (d, 1

H, J_{gem} 11.3 Hz, PhCH_2O), 4.92 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.87 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.65 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.47 (dd, 1 H, $J_{5,6}$ 1.7, J_{gem} 11.1 Hz, H-6a), 4.37 (dd, 1 H, $J_{5,6}$ 4.2, J_{gem} 11.2 Hz, H-6b), 4.21 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 3.63 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 8.8 Hz, H-3), 3.49-3.58 (m, 3 H, H-2, H-4, H-5), 3.55 (s, 3 H, CH_3O), 3.03 (s, 3 H, CH_3S(O)$_2$O), 2.37 (d, 1 H, $J_{1,2}$ 2.2 Hz, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.3 (Ar), 137.5 (Ar), 128.5(8) (Ar), 128.5(4) (Ar), 128.2 (Ar), 128.1 (Ar), 127.9(4) (Ar), 127.8(9) (Ar), 103.7 (C-1), 84.1 (C-3), 76.7 (C-4), 75.2(2) (PhCH_2O), 75.1(7) (PhCH_2O), 74.6 (C-2), 73.1 (C-5), 68.3 (C-6), 57.3 (CH_3O), 37.7 (CH_3S(O)$_2$O); ESI HRMS Calc'd. for $C_{22}H_{28}O_8SNa$ 475.1397. Found 475.1395.

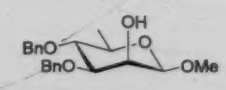


100

Methyl 3,4-di-*O*-benzyl-6-deoxy-β-D-glucopyranoside (100)

To a solution of **99** (370 mg, 0.82 mmol) in DMF (8 mL) was added sodium borohydride (309 mg, 8.2 mmol). The mixture was heated at 80 °C for 3 hours. The reaction was diluted with CH_2Cl_2 and washed with 2% aqueous citric acid, distilled water then brine, dried (Na_2SO_4) and concentrated under reduced pressure. Purification by chromatography over silica gel (1:1, hexanes-EtOAc) gave **100** (235 mg, 80%) as a white solid; R_f 0.48 (1:1, hexanes-EtOAc); $[\alpha]_D$ -16 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.28-7.40 (m, 10 H, ArH), 4.93 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.90 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.87 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.66 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.17 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 3.56 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 9.2 Hz, H-3), 3.55 (s, 3

H, CH_3O), 3.52 (ddd, 1 H, $J_{1,2}$ 7.6, $J_{2,3}$ 9.6, $J_{2,OH}$ 2.0 Hz, H-2), 3.44 (dq, 1 H, $J_{4,5}$ 9.5, $J_{5,6}$ 6.3 Hz, H-5), 3.22 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 8.8 Hz, H-4), 2.35 (d, 1 H, $J_{2,OH}$ 2.1 Hz, OH), 1.34 (d, 3 H, $J_{5,6}$ 6.2 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.6 (Ar), 138.1 (Ar), 128.5 (Ar), 128.4 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 103.5 (C-1), 84.3 (C-3), 82.3 (C-4), 75.3, 75.1, 74.9 (C-2, PhCH_2O×2), 71.5 (C-5), 57.1 (CH_3O), 17.9 (C-6); ESI HRMS Calc'd. for $C_{21}H_{26}O_5Na$ 381.1672. Found 381.1670. Anal. Calc'd. for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 70.68; H, 7.19.

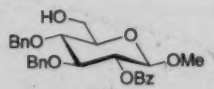


101

Methyl 3,4-di-*O*-benzyl-6-deoxy-β-D-mannopyranoside (101)

Compound **100** (362 mg, 1.01 mmol) was dissolved in a mixture of Me_2SO (6 mL) and acetic anhydride (3 mL). After stirring overnight at room temperature the reaction was concentrated under reduced pressure. The remaining residue was dissolved in a mixture of CH_2Cl_2 (5 mL) and methanol (5 mL) and cooled to 0 °C (ice-water bath). Sodium borohydride (192 mg, 5.05 mmol) was added and the reaction allowed to slowly warm to room temperature. After 3 hours, the reaction was diluted with CH_2Cl_2 and washed with 2% aqueous citric acid, distilled water, brine and dried (Na_2SO_4). The organic phase was then concentrated under reduced pressure. Chromatography over silica gel (7:3, hexanes-EtOAc) yielded **101** (320 mg, 88%) as a white solid; R_f 0.27 (1:1, hexanes-EtOAc); $[\alpha]_D$ -36 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.29-7.39 (m, 10 H, ArH), 4.95 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.77 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O),

4.69 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.66 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.30 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 4.10 (m, 1 H, H-2), 3.51-3.55 (m, 5 H, H-3, H-4, CH_3O), 3.33 (dq, 1 H, $J_{4,5}$ 9.8, $J_{5,6}$ 6.0 Hz, H-5), 2.37 (bs, 1 H, OH), 1.36 (d, 3 H, $J_{5,6}$ 6.2 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4 (Ar), 137.9 (Ar), 128.5 (Ar), 128.4 (Ar), 128.1 (Ar), 127.9(1) (Ar), 127.8(8) (Ar), 127.7 (Ar), 100.6 (C-1), 81.4, 79.7 (C-3, C-4), 75.5 (Ph*C*H$_2$O), 71.4(7), 71.4(6) (C-5, Ph*C*H$_2$O), 68.4 (C-2), 56.9 (*C*H$_3$O), 17.9 (C-6); ESI HRMS Calc'd. for $C_{21}H_{26}O_5Na$ 381.1672. Found 381.1672. Anal. Calc'd. for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 70.59; H, 7.38.

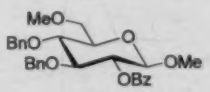


103

Methyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-glucopyranoside (103)

Methyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside[211] **102** (1.28g, 2.68 mmol) in a flame dried flask was dissolved in dry CH_2Cl_2 (20 mL) and cooled to 0 °C (ice-water bath) under argon. A 1.0 M solution of BH_3·THF complex in THF (13.4 mL, 13.43 mmol) was added followed by the dropwise addition of a 1.0 M solution of dibutylboron triflate (1.3 mL, 1.34 mmol). After 2 hours, the reaction was quenched with methanol, neutralized with Et_3N then concentrated under reduced pressure. Chromatography over silica gel (7:3, hexanes-EtOAc) yielded **103** (1.03 g, 80%) as a white solid; R_f 0.29 (1:1, hexanes-EtOAc); $[\alpha]_D$ +39 (*c* 0.65. $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02-8.04 (m, 2 H, ArH), 7.57 (m, 1 H, ArH) 7.43-7.46 (m, 2 H, ArH), 7.29-7.37 (m, 5 H, ArH), 7.14 (bs, 5 H, ArH), 5.25 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.3 Hz,

H-2), 4.88 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.76 (d, 1 H, J_{gem} 11.1 Hz, PhCH_2O), 4.69 (m, 2 H, PhCH_2O), 4.50 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.93 (ddd, 1 H, $J_{6,OH}$ 5.6, $J_{5,6}$ 2.8, J_{gem} 12.0 Hz, H-6a), 3.86 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 9.1 Hz, H-3), 3.78 (ddd, 1 H, $J_{6,OH}$ 8.0, $J_{5,6}$ 4.5, J_{gem} 12.3 Hz, H-6b), 3.73 (dd, 1 H, $J_{3,4}$ 9.0, $J_{4,5}$ 9.5 Hz, H-4), 3.48 (ddd, 1 H, $J_{4,5}$ 9.4, $J_{5,6}$ 2.7, 4.4 Hz, H-5), 3.48 (s, 3 H, CH_3O), 1.93 (dd, 1 H, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2 (*C*=O), 137.8 (Ar), 137.7 (Ar), 133.1 (Ar), 129.9 (Ar), 129.8 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.1 (Ar), 128.0(0) (Ar), 127.9(7) (Ar), 127.7 (Ar), 102.1 (C-1), 82.6 (C-3), 77.7 (C-4), 75.4, 75.1(2), 75.0(8) (C-5, PhCH_2O×2), 73.7 (C-2), 61.9 (C-6), 57.0 (CH_3O); ESI HRMS Calc'd. for $C_{28}H_{30}O_7Na$ 501.1884. Found 501.1886; Anal. Calc'd. for $C_{28}H_{30}O_7$: C, 70.28; H, 6.32. Found: C, 70.30; H, 6.45.

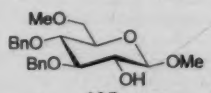


104

Methyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-methyl-β-D-glucopyranoside (104)

To a solution of compound **103** (953 mg, 1.99 mmol) in dry DMF (20 mL) was added methyl iodide (248 μL, 3.98 mmol). The reaction was cooled to 0 °C (ice-water bath) under argon before the addition of sodium hydride (159 mg, 3.98 mmol). After 3 hours stirring at 0 °C, the reaction was quenched with acetic acid then concentrated under reduced pressure. Purification by chromatography over silica gel (4:1, hexanes-EtOAc) gave **104** (937 mg, 96%) as a white solid; R_f 0.51 (1:1, hexanes-EtOAc); $[\alpha]_D$ +34 (*c* 0.27, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02-8.04 (m, 2 H, ArH), 7.57 (m, 1 H, ArH), 7.42-7.45 (m, 2 H, ArH), 7.29-7.37 (m, 5 H, ArH), 7.13 (bs, 5 H, ArH), 5.27 (dd, 1

H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.2 Hz, H-2), 4.86 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.75 (d, 1 H, J_{gem} 11.1 Hz, PhCH_2O), 4.64-4.69 (m, 2 H, PhCH_2O), 4.45 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 3.84 (dd, 1 H, $J_{2,3} \approx J_{3,4}$ 9.1 Hz, H-3), 3.76 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.4 Hz, H-4), 3.70 (ABX, 1 H, $J_{5,6}$ 4.6, J_{gem} 10.8 Hz, H-6a), 3.66 (ABX, 1 H, $J_{5,6}$ 1.9, J_{gem} 10.8 Hz, H-6b), 3.53 (ABX, 1 H, $J_{4,5}$ 9.7, $J_{5,6}$ 1.9, 4.6 Hz, H-5), 3.47 (s, 3 H, CH_3O), 3.42 (s, 3 H, CH_3O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2 (*C*=O), 138.1 (Ar), 137.8 (Ar), 133.0 (Ar), 130.0 (Ar), 129.8 (Ar), 128.5 (Ar), 128.3 (Ar), 128.2 (Ar), 128.0 (Ar), 127.9(9) (Ar), 127.9(5) (Ar), 127.6 (Ar), 102.0 (C-1), 82.8 (C-3), 77.9 (C-4), 75.1, 75.0(4), 74.9(8) (C-5, PhCH_2O×2), 73.7 (C-2), 71.2 (C-6), 59.5 (*C*H_3O), 56.7 (*C*H_3O); ESI HRMS Calc'd. for $C_{29}H_{32}O_7Na$ 515.2040. Found 515.2042; Anal. Calc'd. for $C_{29}H_{32}O_7$: C, 70.71; H, 6.55. Found: C, 70.82; H, 6.64.

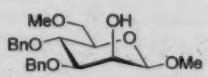


105

Methyl 3,4-di-*O*-benzyl-6-*O*-methyl-β-D-glucopyranoside (105)

Compound **104** (937 mg, 1.90 mmol) was dissolved in a mixture of CH_2Cl_2 (10 mL) and methanol (10 mL). A 0.5 M solution of sodium methoxide in methanol (5 mL) was added then the reaction was stirred at room temperature overnight. The reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered and the filtrate concentrated under reduced pressure. Chromatography over silica gel (1:1, hexanes-EtOAc) gave **105** (663 mg, 90%) as a white powdery solid; R_f 0.31 (1:1, hexanes-EtOAc); $[\alpha]_D$ -15 (*c* 0.59, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26-7.39 (m, 10 H, ArH), 4.92 (d, 1 H, J_{gem} 11.4 Hz, PhCH_2O), 4.88 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.87 (d,

1 H, J_{gem} 11.4 Hz, PhCH_2O), 4.63 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.18 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 3.66 (dd, 1 H, $J_{5,6}$ 2.2, J_{gem} 10.8, H-6a), 3.57-3.63 (m, 3 H, H-3, H-4, H-6b), 3.56 (s, 3 H, CH_3O), 3.53 (ddd, 1 H, $J_{2,OH}$ 2.0, $J_{1,2} \approx J_{2,3}$ 7.9 Hz, H-2), 3.45 (ddd, 1 H, $J_{4,5}$ 9.3, $J_{5,6}$ 2.1, 4.1 Hz, H-5), 3.39 (s, 3 H, CH_3O), 2.35 (d, 1 H, $J_{2,OH}$ 2.1 Hz, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.6 (Ar), 138.2 (Ar), 128.5 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 103.7 (C-1), 84.4 (C-3), 77.5 (C-4), 75.1, 75.0(2), 75.0(0) (C-5, PhCH_2O×2), 74.6 (C-2), 71.2 (C-6), 59.4 (CH_3O), 57.2 (CH_3O); ESI HRMS Calc'd. for $C_{22}H_{28}O_6Na$ 411.1778. Found 411.1775; Anal. Calc'd. for $C_{22}H_{28}O_6$: C, 68.02; H, 7.27. Found: C, 68.11; H, 7.39.

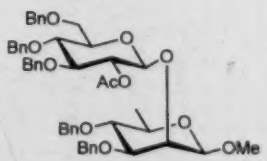


106

Methyl 3,4-di-*O*-benzyl-6-*O*-methyl-β-D-mannopyranoside (106)

Compound **105** (600 mg, 1.54 mmol) was dissolved in a mixture of Me_2SO (5 mL) and acetic anhydride (5 mL). After stirring overnight at room temperature the reaction was concentrated under reduced pressure. The remaining residue was dissolved in a mixture of CH_2Cl_2 (5 mL) and methanol (5 mL) and cooled to 0 °C (ice-water bath). Sodium borohydride (294 mg, 7.72 mmol) was added and the reaction allowed to slowly warm to room temperature. After 3 hours, the reaction was diluted with CH_2Cl_2 and washed with 2% aqueous citric acid, distilled water, brine and dried (Na_2SO_4). The organic phase was then concentrated under reduced pressure. Chromatography over silica gel (7:3, hexanes-EtOAc) yielded **106** (508 mg, 85%) as a white solid; R_f 0.14 (1:1,

hexanes-EtOAc); $[\alpha]_D$ -27 (*c* 0.6, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.27-7.39 (m, 10 H, ArH), 4.93 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.78 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.69 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.62 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.32 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 4.09 (m, 1 H, $J_{1,2}$ 0.9, $J_{2,3}$ 3.0 Hz, H-2), 3.88 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.4 Hz, H-4), 3.68 (dd, 1 H, $J_{5,6}$ 2.2, J_{gem} 10.7 Hz, H-6a), 3.63 (dd, 1 H, $J_{5,6}$ 5.0, J_{gem} 10.6 Hz, H-6b), 3.57 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.1 Hz, H-3), 3.54 (s, 3 H, CH_3O), 3.39 (s, 3 H, CH_3O), 3.38 (ddd, 1 H, $J_{4,5}$ 9.6, $J_{5,6}$ 2.1, 4.9 Hz, H-5), 2.38 (d, 1 H, $J_{2,OH}$ 2.3 Hz, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4 (Ar), 137.9 (Ar), 128.5 (Ar), 128.4 (Ar), 128.1 (Ar), 127.8(8) (Ar), 127.8(4) (Ar), 127.7(6) (Ar), 100.8 (C-1), 81.4 (C-3), 75.2(1) (PhCH_2O), 75.1(5) (C-5), 74.2 (C-4), 71.5(4), 71.4(7) (C-6, PhCH_2O), 68.3 (C-2), 59.4 (CH_3O), 56.9 (CH_3O); ESI HRMS Calc'd. for $C_{22}H_{28}O_6Na$ 411.1778. Found 411.1782.

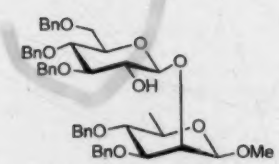


107

Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl (1→2) 3,4-di-*O*-benzyl-6-deoxy-β-D-mannopyranoside (107)

Monosaccharide acceptor **101** (250 mg, 0.70 mmol) was reacted with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate[175] **21** (533 mg, 0.84 mmol) in CH_2Cl_2 (5 mL) using TMSOTf (8 μL, 0.04 mmol) under argon at 0 °C then processed as described for **22**. The product was purified by chromatography over silica gel (4:1, hexanes-EtOAc) to give **107** (560 mg, 96%) as a colourless syrup; R_f 0.65 (1:1, hexanes-

EtOAc); $[\alpha]_D$ -43 (*c* 0.8, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.19-7.39, (m, 25 H, ArH), 5.13 (dd, 1 H, *J* 8.1, 9.5 Hz, H-2′), 4.99 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.88 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.82-4.84 (m, 2 H, H-1′, PhCH_2O), 4.80 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.76 (d, 1 H, J_{gem} 11.4 Hz, PhCH_2O), 4.59 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.52-4.57 (m, 2 H, PhCH_2O), 4.51 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O), 4.48 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.23 (d, 1 H, $J_{2,3}$ 2.8 Hz, H-2), 4.15 (s, 1 H, H-1), 3.74-3.79 (m, 2 H, H-3′, H-6a′), 3.55-3.67 (m, 3 H, H-4′, H-5′, H-6b′), 3.48 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.1 Hz, H-4), 3.43 (s, 3 H, CH_3O), 3.41 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3), 3.24 (dq, 1 H, $J_{4,5}$ 9.1, $J_{5,6}$ 6.0 Hz, H-5), 2.03 (s, 3 H, CH_3C(O)O), 1.32 (d, 3 H, $J_{5,6}$ 6.0 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8 (*C*=O), 138.8 (Ar), 138.6 (Ar), 138.3 (Ar), 138.1 (Ar), 128.4 (Ar), 128.3(4) (Ar), 128.3(0) (Ar), 128.2 (Ar), 128.1(2) (Ar), 128.1(0) (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (Ar), 101.7 (C-1), 101.1 (C-1′), 83.1 (C-3′), 79.9, 79.8 (C-3, C-4), 78.1 (C-4′), 75.5 (Ph*C*H$_2$O), 75.0, 74.8, 74.7 (C-5′, Ph*C*H$_2$O×2), 73.6, 73.5 (C-2′, PhCH$_2$O), 72.5 (C-2), 71.7 (C-5), 69.9, 69.8 (C-6, Ph*C*H$_2$O), 56.5 (*C*H$_3$O), 21.1 (*C*H$_3$C(O)O), 18.0 (C-6); ESI HRMS Calc'd. for $C_{50}H_{56}O_{11}$N16a 855.3715. Found 855.3715.

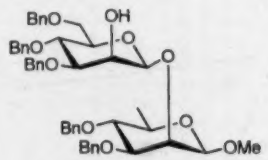


108

Methyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl (1→2) 3,4-di-*O*-benzyl-6-deoxy-β-D-mannopyranoside (108)

Disaccharide **107** (529 mg, 0.63 mmol) was dissolved in CH_2Cl_2-methanol (1:1, 20 mL) and treated with 0.5 M MeONa/MeOH (5 mL) then processed as described for **23**. Purification of the product by chromatography over silica gel (7:3, hexanes-EtOAc) gave **108** (502 mg, quant.) as a colourless syrup; R_f 0.49 (1:1, hexanes-EtOAc); $[\alpha]_D$ -45 (*c* 0.57, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24-7.44 (m, 23 H, ArH), 7.17-7.19 (m, 2 H, ArH), 5.09 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 5.00 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.90 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.87 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.82 (d, 1 H, J_{gem} 11.3 Hz, PhCH_2O), 4.61 (d, 1 H, J_{gem} 10.8 Hz, PhCH_2O), 4.59 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.56 (d, 1 H, J_{gem} 12.4 Hz, PhCH_2O), 4.54 (d, 1 H, J_{gem} 11.1 Hz, PhCH_2O), 4.49 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.45 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.27 (s, 1 H, H-1), 4.22 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.75 (dd, 1 H, $J_{2',3'}$ 9.0 Hz, H-2′), 3.64-3.72 (m, 3 H, H-3′, H-6a′, H-6b′), 3.52-3.59 (m, 3 H, H-4′, H-5′, H-4), 3.52 (s, 3 H, CH_3O), 3.49 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.3 Hz, H-3), 3.31 (dq, 1 H, $J_{4,5}$ 9.0, $J_{5,6}$ 6.1 Hz, H-5), 1.37 (d, 3 H, $J_{5,6}$ 6.1 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.1 (Ar), 138.6 (Ar), 138.3 (Ar), 138.2 (Ar), 138.1 (Ar), 128.3(4) (Ar), 128.3(0) (Ar), 128.2(8) (Ar), 128.0(7) (Ar), 128.0(6) (Ar), 127.9(6) (Ar), 127.8 (Ar), 127.6(8) (Ar), 127.6(5) (Ar), 127.5(4) (Ar), 127.5(3) (Ar), 127.4 (Ar), 104.7 (C-1′), 101.3 (C-1), 85.3 (C-3′), 80.1, 79.9 (C-3, C-4), 77.3 (C-4′, C-5′),

75.5(3), 75.5(2), 75.5(0) (C-2, C-2′, $PhCH_2O$), 75.3 ($PhCH_2O$), 75.0 ($PhCH_2O$), 74.8 (C-4′, C-5′), 73.4 ($PhCH_2O$), 71.8 (C-5), 70.4 ($PhCH_2O$), 69.7 (C-6′), 57.0 (CH_3O), 18.0 (C-6); ESI HRMS Calc'd. for $C_{48}H_{54}O_{10}Na$ 813.3609. Found 813.3609.

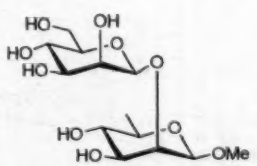


109

Methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl (1→2) 3,4-di-*O*-benzyl-6-deoxy-β-D-mannopyranoside (109)

As described for compound **24**, disaccharide **108** (475 mg, 0.60 mmol) was dissolved in freshly distilled Me_2SO (10 mL) and acetic anhydride (5 mL). The concentrated reaction mixture was then treated with 1.0 M L-Selectride® in THF (2.4 mL, 2.40 mmol) in dry THF (10 mL) at -78 °C under argon. Purification by column chromatography over silica gel (1:1, hexanes-EtOAc) gave **109** (390 mg, 82%) as a white solid; R_f 0.41 (1:1, hexanes-EtOAc); $[\alpha]_D$ -52 (*c* 0.71, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.22-7.24 (m, 25 H, ArH), 4.96 (d, 1 H, J_{gem} 11.3 Hz, $PhCH_2O$), 4.94 (d, 1 H, J_{gem} 12.2 Hz, $PhCH_2O$), 4.93 (d, 1 H, J_{gem} 10.9 Hz, $PhCH_2O$), 4.90 (d, 1 H, $J_{1',2'}$ 0.7 Hz, H-1′), 4.85 (d, 1 H, J_{gem} 12.0 Hz, $PhCH_2O$), 4.65 (d, 1 H, J_{gem} 12.1 Hz, $PhCH_2O$), 4.60 (d, 1 H, J_{gem} 10.8 Hz, $PhCH_2O$), 4.56 (d, 1 H, J_{gem} 11.0 Hz, $PhCH_2O$), 4.50 (d, 1 H, $J_{2,3}$ 2.8 Hz, H-2), 4.46 (d, 1 H, J_{gem} 11.4 Hz, $PhCH_2O$), 4.45 (d, 1 H, J_{gem} 12.0 Hz, $PhCH_2O$), 4.42 (d, 1 H, J_{gem} 12.0 Hz, $PhCH_2O$), 4.34 (d, 1 H, $J_{2',3'}$ 2.8 Hz, H-2′), 4.26 (s, 1 H, H-1), 3.91 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.3 Hz, H-4′), 3.76 (dd, 1 H, $J_{5',6'}$ 2.0, J_{gem} 10.6 Hz, H-6a′), 3.63

(dd, 1 H, $J_{5',6'}$ 6.1, J_{gem} 10.5 Hz, H-6b'), 3.58 (dd, 1 H, H-3'), 3.44-3.52 (m, 3 H, H-5', H-3, H-4), 3.48 (s, 3 H, CH_3O), 3.31 (dq, 1 H, $J_{4,5}$ 8.7, $J_{5,6}$ 6.0 Hz, H-5), 1.37 (d, 3 H, $J_{5,6}$ 6.1 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4(9) (Ar), 138.4(5) (Ar), 138.2 (Ar), 138.1 (Ar), 128.4 (Ar), 128.3(1) (Ar), 128.2(8) (Ar), 128.2(1) (Ar), 128.2(0) (Ar), 128.1 (Ar), 127.9(Ar), 127.7 (Ar), 127.6(4) (Ar), 127.6(3) (Ar), 127.5(5) (Ar), 127.5(0) (Ar), 102.0 (C-1), 99.1 (C-1'), 81.4 (C-3'), 80.1 (C-3/C-5'), 79.4 (C-4), 75.4 (PhCH_2O), 75.1 (PhCH_2O), 75.0 (C-3/C-5'), 74.4 (C-4'), 73.4 (PhCH_2O), 71.8 (C-5), 70.7 (PhCH_2O), 70.4, 70.1, 70.0 (C-2, C-6', PhCH_2O), 67.7 (C-2'), 57.1 (CH_3O), 18.0 (C-6); ESI HRMS Calc'd. for $C_{48}H_{54}O_{10}Na$ 813.3609. Found 813.3614. Anal. Calc'd. for $C_{48}H_{54}O_{10}$: C, 71.90; H, 6.21. Found: C, 71.95; H, 6.22.

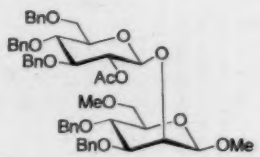


16

Methyl β-D-mannopyranosyl (1→2) 6-deoxy-β-D-mannopyranoside (16)

Compound **109** (55.0 mg, 0.070 mmol) was dissolved in CH_2Cl_2 (5 mL) and methanol (5 mL) then stirred with 10% Pd/C (50 mg) under an H_2 atmosphere then processed as described for **1**. Filtration then lyophilization gave **16** (20.4 mg, 86%) as a clear glass; R_f 0.49 (6:3.5:0.5, CH_2Cl_2-methanol-H_2O); $[\alpha]_D$ -45 (*c* 1.1, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 4.82 (s, 1 H, H-1'), 4.61 (s, 1 H, H-1), 4.24 (d, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 4.10 (d, 1 H, $J_{2',3'}$ 3.3 Hz, H-2'), 3.92 (dd, 1 H, $J_{5',6'}$ 2.3, J_{gem} 12.3 Hz, H-6a'), 3.73 (dd, 1 H, $J_{5',6'}$ 6.8, J_{gem} 12.3 Hz, H-6b'), 3.63 (dd, 1 H, $J_{2',3'}$ 3.3, $J_{3',4'}$ 9.3 Hz, H-3'), 3.58 (dd, 1 H,

$J_{2,3}$ 3.3, $J_{3,4}$ 9.3 Hz, H-3) 3.55 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.7 Hz, H-4′), 3.51 (s, 3 H, CH_3O), 3.34-3.42 (m, 3 H, H-5′, H-4, H-5), 1.32 (d, 3 H, $J_{5,6}$ 5.6 Hz, CH_3); ^{13}C NMR (125 MHz, D_2O) δ 102.1 (C-1, $^1J_{C\text{-}1,H\text{-}1}$ 152.3 Hz, β), 101.3 (C-1′, $^1J_{C\text{-}1',H\text{-}1'}$ 162.7 Hz, β), 78.6 (C-2), 77.2 (C-5′), 73.7, 73.4, 73.2, 72.9 (C-3′, C-3, C-4, C-5), 71.2 (C-2′), 67.7 (C-4′), 62.0 (C-6′), 57.9 (CH_3O), 17.5 (C-6); ESI HRMS Calc'd. for $C_{13}H_{24}O_{10}Na$ 363.1262. Found 363.1261.

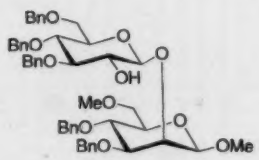


110

Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl (1→2) 3,4-di-*O*-benzyl-6-*O*-methyl-β-D-mannopyranoside (110)

Monosaccharide acceptor **106** (198 mg, 0.51 mmol) was reacted with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate[175] **21** (390 mg, 0.61 mmol) in CH_2Cl_2 (5 mL) using TMSOTf (6 μL, 0.03 mmol) under argon at 0 °C then processed as described for **1**. The product was purified by chromatography over silica gel (4:1, hexanes-EtOAc) to give **110** (298 mg, 68%) as a colourless syrup; R_f 0.43 (1:1, hexanes-EtOAc); $[\alpha]_D$ -43 (*c* 0.47, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18-7.37 (m, 25 H, ArH), 5.12 (dd, 1 H, $J_{1',2'}$ 8.1, $J_{2',3'}$ 9.5 Hz, H-2′), 4.96 (d, 1 H, J_{gem} 11.1 Hz, PhCH_2O), 4.89 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.82 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.82 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.79 (d, 1 H, J_{gem} 11.6 Hz, PhCH_2O), 4.75 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.55 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.53 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O),

4.52 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.48 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.46 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.23 (d, 1 H, $J_{2,3}$ 2.9 Hz, H-2), 4.19 (s, 1 H, H-1), 3.76 (dd, 1 H, $J_{2',3'}$ 8.2, $J_{3',4'}$ 9.4 Hz, H-3′), 3.74 (dd, 1 H, $J_{5',6'}$ 1.4, J_{gem} 8.9 Hz, H-6a′), 3.53-3.66 (m, 6 H, H-4′, H-5′, H-6b′, H-4, H-6a, H-6b), 3.46-3.48 (m, 4 H, H-3, CH_3O), 3.36-3.39 (m, 4 H, H-5, CH_3O), 2.03 (s, 3 H, CH_3C(O)O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8 (*C*=O), 138.7 (Ar), 138.5 (Ar), 138.2 (Ar), 138.1 (Ar), 138.0 (Ar), 128.4 (Ar), 128.3(3) (Ar), 128.3(0) (Ar), 128.2(5) (Ar), 128.1(3) (Ar), 128.0(9) (Ar), 128.0 (Ar), 127.8 (Ar), 127.7(2) (Ar), 127.7(1) (Ar), 127.6 (Ar), 127.5(5) (Ar), 127.4(8) (Ar), 101.7 (C-1), 101.1 (C-1′), 83.2 (C-3′), 80.0 (C-3), 78.1 (C-4′/C-5′/C-4), 75.5, 75.2, 74.9, 74.8(2), 74.7(5) (C-4′/C-5′/C-4, C-5, Ph*C*H_2O×3), 73.6, 73.5, 72.8, 72.2 (C-2, C-6, C-2′, Ph*C*H_2O), 69.8(4), 69.8(0) (C-6′, Ph*C*H_2O), 59.3 (*C*H_3O), 56.7 (*C*H_3O), 21.1 (*C*H_3C(O)O); ESI HRMS Calc'd. for $C_{51}H_{58}O_{12}Na$ 885.3821. Found 885.3811.

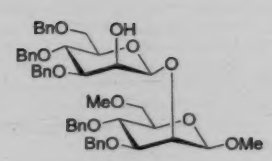


111

Methyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl (1→2) 3,4-di-*O*-benzyl-6-*O*-methyl-β-D-mannopyranoside (111)

Disaccharide **110** (281 mg, 0.33 mmol) was dissolved in CH_2Cl_2-methanol (1:1, 10 mL) and treated with 0.5 M MeONa/MeOH (3 mL) then processed as described for **23**. Purification of the product by chromatography over silica gel (7:3, hexanes-EtOAc) gave **111** (257 mg, 96%): R_f 0.31 (1:1, hexanes-EtOAc); $[\alpha]_D$ -42 (*c* 0.80, $CHCl_3$); ^{1}H

NMR (500 MHz, $CDCl_3$) δ 7.23-7.43 (m, 23 H, ArH), 7.16-7.18 (m, 2 H, ArH), 5.08 (d, 1 H, J_{gem} 11.2 Hz, PhCH_2O), 4.97 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.90 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.86 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.80 (d, 1 H, J_{gem} 11.4 Hz, PhCH_2O), 4.61 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.57 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.55 (d, 1 H, J_{gem} 12.2 Hz, PhCH_2O), 4.53 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.48 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.44 (d, 1 H, J_{gem} 12.0 Hz, PhCH_2O), 4.28 (s, 1 H, H-1), 4.23 (d, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 3.84 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4), 3.73 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 8.9 Hz, H-2'), 3.61-3.71 (m, 5 H, H-3', H-6a', H-6b', H-6a, H-6b), 3.51-3.55 (m, 3 H, H-4', H-3, H-4), 3.53 (s, 3 H, CH_3O), 3.39 (s, 3 H, CH_3O), 3.37 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6}$ 2.0, 5.2 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.1 (Ar), 138.5 (Ar), 138.2 (Ar), 138.1(3) (Ar), 138.1(1) (Ar), 128.3(5) (Ar), 128.3(2) (Ar), 128.3(0) (Ar), 128.2(5) (Ar), 128.1 (Ar), 128.0(4) (Ar), 128.0(3) (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (Ar), 104.4 (C-1'), 101.6 (C-1), 85.2 (C-3'), 80.2 (C-3), 77.3 (C-4'/C-5') 75.6, 75.5, 75.3, 75.0, 74.9, 74.8 (C-2', C-4'/C-5', C-2, C-5, PhCH_2O×3), 74.4 (C-4), 73.4 (PhCH_2O), 71.7 (C-6), 70.4 (PhCH_2O), 69.7 (C-6'), 59.4 (CH_3O), 57.2 (CH_3O); ESI HRMS Calc'd. for $C_{49}H_{56}O_{11}Na$ 843.3715. Found 843.3718.

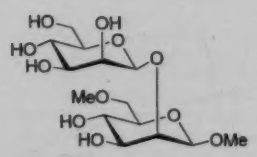


112

Methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl (1→2) 3,4-di-*O*-benzyl-6-*O*-methyl-β-D-mannopyranoside (112)

As described for compound **24**, disaccharide **111** (257 mg, 0.31 mmol) was dissolved in freshly distilled Me_2SO (8 mL) and acetic anhydride (4 mL). The concentrated reaction mixture was then treated with 1.0 M L-Selectride® in THF (1.3 mL, 1.25 mmol) in dry THF (10 mL) at -78 °C under argon. Purification by column chromatography over silica gel (7:3, hexanes-EtOAc) gave **112** (200 mg, 78%) as a white solid; R_f 0.25 (1:1, hexanes-EtOAc); $[\alpha]_D$ -49 (*c* 0.62, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37-7.41 (m, 4 H, ArH), 7.21-7.34 (m, 21 H, ArH), 4.96 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.93 (d, 1 H, J_{gem} 10.7 Hz, PhCH_2O), 4.91 (d, 1 H, $J_{1',2'}$ 0.8 Hz, H-1′), 4.91 (d, 1 H, J_{gem} 10.0 Hz, PhCH_2O), 4.84 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.64 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.56 (d, 1 H, J_{gem} 10.9 Hz, PhCH_2O), 4.55 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.50 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 4.46 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.45 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 4.42 (d, 1 H, J_{gem} 11.9 Hz, PhCH_2O), 4.34 (d, 1 H, $J_{2',3'}$ 2.9 Hz, H-2′), 4.27 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1), 3.90 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.3 Hz, H-4′), 3.80 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.6 Hz, H-4), 3.76 (dd, 1 H, $J_{5',6'}$ 2.0, J_{gem} 10.6 Hz, H-6a′), 3.67 (dd, 1 H, $J_{5,6}$ 2.0, J_{gem} 10.6 Hz, H-6a), 3.62 (dd, 1 H, $J_{5',6'}$ 6.0, J_{gem} 10.7 Hz, H-6b′), 3.61 (dd, 1 H, $J_{5,6}$ 5.2, J_{gem} 10.7 Hz, H-6b), 3.56 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.1 Hz, H-3), 3.54 (dd, 1 H, $J_{2',3'}$ 3.[illegible], $J_{3',4'}$ 9.3 Hz, H-3′), 3.49 (ddd, 1 H, $J_{4',5'}$ 9.7, $J_{5',6'}$ 1.9, 6.1 Hz, H-5′), 3.48 (s, 3 H,

CH_3O), 3.38 (s, 3 H, CH_3O), 3.36 (ddd, 1 H, $J_{4,5}$ 9.8, $J_{5,6}$ 2.0, 5.2 Hz, H-5), 2.78 (bs, 1 H, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4(8) (Ar), 138.4(6) (Ar), 138.2 (Ar), 138.1(4) (Ar), 138.1(1) (Ar), 128.4 (Ar), 128.3(2) (Ar), 128.3(0) (Ar), 128.2(9) (Ar), 128.2(7) (Ar), 128.2 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.7 (Ar), 127.6(4) (Ar), 127.6(1) (Ar), 127.5(2) (Ar), 127.4(9) (Ar), 102.2 (C-1), 99.0 (C-1′), 81.4 (C-3), 80.2 (C-3′), 75.3, 75.2, 75.1, 75.0 (C-5, C-5′, PhCH_2O×2), 74.4 (C-4′), 73.9 (C-4), 73.4 (PhCH_2O), 71.6 (C-6), 70.6 (PhCH_2O), 70.1, 70.0 (C-6′, PhCH_2O), 67.7 (C-2′), 59.2 (CH_3O), 57.2 (CH_3O); ESI HRMS Calc'd. for $C_{49}H_{56}O_{11}Na$ 843.3715. Found 843.3705.

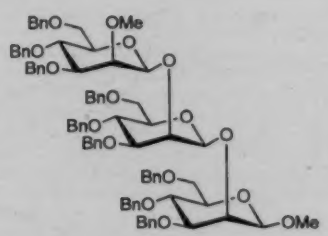


17

Methyl β-D-mannopyranosyl (1→2) 6-*O*-methyl-β-D-mannopyranoside (17)

Compound **112** (50.0 mg, 0.061 mmol) was dissolved in CH_2Cl_2 (5 mL)-methanol (5 mL) and stirred with 10% Pd/C (50 mg) under an H_2 atmosphere then processed as described for **1**. Filtration then lyophilization gave **17** (15.6 mg, 69%) as a clear glass; R_f 0.33 (6:3.5:0.5, CH_2Cl_2-MeOH-H_2O); $[\alpha]_D$ -73 (*c* 0.25, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 4.82 (d, 1 H, $J_{1',2'}$ 0.8 Hz, H-1′), 4.63 (s, 1 H, H-1), 4.25 (d, 1 H, $J_{2,3}$ 2.9 Hz, H-2), 4.11 (dd, 1 H, $J_{1',2'}$ 0.7, $J_{2',3'}$ 3.3 Hz, H-2′), 3.92 (dd, 1 H, $J_{5',6'}$ 2.3, J_{gem} 12.3 Hz, H-6a′), 3.80 (dd, 1 H, $J_{5,6}$ 2.1, J_{gem} 11.2 Hz, H-6a), 3.73 (dd, 1 H, $J_{5',6'}$ 6.7, J_{gem} 12.3 Hz, H-6b′), 3.67 (dd, 1 H, $J_{5,6}$ 6.3, J_{gem} 11.3 Hz, H-6b), 3.62-3.64 (m, 2 H, H-3′, H-3), 3.59 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.3 Hz, H-4), 3.56 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.7 Hz, H-4′), 3.53 (s, 3 H, CH_3O-C1), 3.49

(ddd, 1 H, $J_{4,5}$ 9.2, $J_{5,6}$ 2.2, 6.3 Hz, H-5), 3.42 (s, 3 H, CH_3O-C6), 3.36 (ddd, 1 H, $J_{4',5'}$ 9.7, $J_{5',6'}$ 2.2, 6.6 Hz, H-5'); ^{13}C NMR (125 MHz, D_2O) δ 102.3 (C-1, $^1J_{C1,H1}$ 160.1 Hz, β), 101.3 (C-1', $^1J_{C1,H1}$ 162.4 Hz, β), 78.5 (C-2), 77.2 (C-5'), 75.8 (C-5), 73.6, 73.1 (C-3', C-3), 72.1 (C-6), 71.2 (C-2'), 68.1 (C-4), 67.7 (C-4'), 62.0 (C-6'), 59.4 (CH_3OC-6), 58.1 (CH_3OC-1); ESI HRMS Calc'd. for $C_{14}H_{26}O_{11}Na$ 393.1367. Found 393.1365.

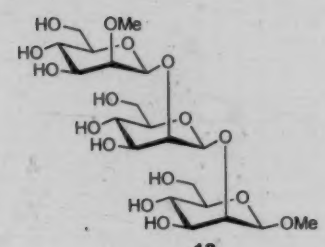


113

Methyl 3,4,6-tri-*O*-benzyl-2-*O*-methyl-β-D-mannopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl (1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (113)

Compound **27** (49 mg, 0.037 mmol) was dissolved in dry DMF (0.5 mL) and the solution was cooled to 0 °C (ice-water bath). Methyl iodide (12 μL, 0.18 mmol) and sodium hydride (7 mg, 0.18 mmol) were added and the reaction was stirred at 0 °C (ice-water bath). After two hours, the reaction was quenched with methanol and concentrated under reduced pressure. The residue was subjected to column chromatography over silica gel (95:5, toluene-EtOAc) to give **113** (45 mg, 91%) as a clear syrup; R_f 0.50 (1:1, hexanes-EtOAc); $[\alpha]_D$ -67 (*c* 0.38, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45-7.47 (m, 4 H, ArH), 7.10-7.36 (m, 39 H, ArH), 6.95-6.97 (m, 2 H, ArH), 5.18 (s, 1 H, H-1), 5.09 (s, 1 H, H-1), 5.07 (d, 1 H, J_{gem} 12.1 Hz, PhCH_2O), 5.04 (d, 1 H, J_{gem} 11.4 Hz, PhCH_2O), 5.02 (d, 1 H, J_{gem} 11.5 Hz, PhCH_2O), 4.87 (m, 2 H, PhCH_2O), 4.72 (d, 1 H, J_{gem} 12.3 Hz,

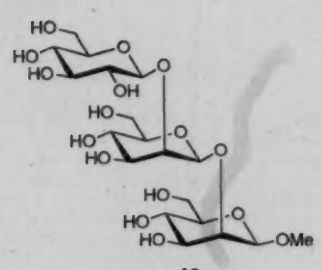
PhCH_2O), 4.67 (d, 1 H, $J_{2',3'}$ 3.1 Hz, H-2'), 4.62 (d, 1 H, $J_{2,3}$ 3.4 Hz, H-2), 4.59 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2O), 4.45-4.58 (m, 8 H, PhCH_2O×8), 4.34 (s, 1 H, H-1), 4.30 (d, 1 H, J_{gem} 10.0 Hz, PhCH_2O), 4.28 (d, 1 H, J_{gem} 11.4 Hz, PhCH_2O), 4.17 (d, 1 H, J_{gem} 11.6 Hz, PhCH_2O), 3.95 (d, 1 H, $J_{2'',3''}$ 3.0 Hz, H-2''), 3.93 (dd, 1 H, $J_{3',4'} \approx J_{4',5'}$ 9.5 Hz, H-4'), 3.87 (dd, 1 H, $J_{5',6'}$ 1.6, J_{gem} 10.6 Hz, H-6a'), 3.71-3.79 (m, 7 H, H-4'', H-6a'', H-6b'', H-6b', H-4, H-6a, H-6b), 3.62-3.65 (m, 2 H, H-3', H-3), 3.64 (s, 3 H, CH_3OC-2''), 3.54-3.59 (m, 3 H, H-3'', H-5', H-5), 3.53 (s, 3 H, CH_3OC-1), 3.46 (ddd, 1 H, $J_{4',5'}$ 9.6, $J_{5',6'}$ 3.0, 3.0 Hz, H-5'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.7, 138.6, 138.5, 138.3(2), 138.2(7), 138.0(2), 138.0(1), 137.9, 128.6, 128.4, 128.3(1), 128.3(0), 128.2(8), 128.2(3), 128.1(9), 128.1(8), 128.1(5), 128.1(2), 128.1(0), 128.0(8), 128.0, 127.7, 127.6, 127.4(9), 127.4(7), 127.3(1), 127.2(7), 127.2(4), 127.1(5), 102.7, 101.8, 100.3, 83.4, 80.6, 80.0, 76.5, 75.5, 75.3, 75.2(4), 75.1(6), 75.0(8), 75.0(6), 74.9(7), 74.7, 74.4, 73.6, 73.4, 73.3, 71.7, 70.8, 70.3(3), 70.2(5), 69.9, 69.4, 69.1, 68.8, 61.3, 57.3; ESI HRMS Calc'd. for $C_{83}H_{90}O_{16}Na$ 1365.6124. Found 1365.6121.



18

Methyl 2-*O*-methyl-β-D-mannopyranosyl (1→2) β-D-mannopyranosyl (1→2) β-D-mannopyranoside (18)

Compound **113** (44.9 mg, 0.033 mmol) was dissolved in CH_2Cl_2 (5 mL)-methanol (5 mL) and stirred with 10% Pd/C (50 mg) under a H_2 atmosphere then processed as described for **1**. Filtration then lyophilization gave **18** (17.0 mg, 95%) as a clear glass; R_f 0.14 (6:3.5:0.5, CH_2Cl_2-MeOH-H_2O); $[\alpha]_D$ -86 (*c* 0.87, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 4.99 (s, 1 H, H-1″), 4.88 (s, 1 H, H-1′), 4.64 (s, 1 H, H-1), 4.32 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2′), 4.24 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 3.90-3.96 (m, 3 H, H-6a″, H-6a′, H-6a), 3.88 (d, 1 H, $J_{2,3}$ 3.3 Hz, H-2″), 3.57-3.75 (m, 7 H, H-3″, H-6b″, H-3′, H-4′, H-6b′, H-3, H-6b), 3.61 (s, 3 H, CH_3OC-2″), 3.55 (s, 3 H, CH_3OC-1), 3.52 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.7 Hz, H-4), 3.46 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4″), 3.32-3.40 (m, 3 H, H-5″, H-5′, H-5); ^{13}C NMR (125 MHz, D_2O) δ 102.2 (C-1, $^1J_{C1,H1}$ 160.2 Hz, β), 102.1 (C-1″, $^1J_{C1,H1}$ 162.8 Hz, β), 101.9 (C-1′, $^1J_{C1,H1}$ 162.4 Hz, β), 81.4 (C-2″), 79.5 (C-2), 78.8 (C-2′), 77.2 (×2), 77.1 (C-5″, C-5′, C-5), 73.9 (C-3″), 73.0, 72.9 (C-3′, C-3), 68.5 (C-3″), 68.1, 68.0 (C-4″, C-4), 62.6 (CH_3OC-2″), 62.1, 61.7, 61.6 (C-6″, C-6′, C-6), 57.9 (CH_3OC-1); ESI HRMS Calc'd. for $C_{20}H_{36}O_{16}Na$ 555.1896. Found 555.1889.



19

Methyl β-D-glucopyranosyl (1→2) β-D-mannopyranosyl (1→2) β-D-mannopyranoside (19)

Compound **26** (54.8 mg, 0.041 mmol) was dissolved in CH_2Cl_2 (5 mL)-methanol (5 mL) and stirred with 10% Pd/C (50 mg) under an H_2 atmosphere then processed as described for **1**. Filtration then lyophilization gave **19** (19.3 mg, 90%) as a clear glass; R_f 0.06 (6:3.5:0.5, CH_2Cl_2-MeOH-H_2O); $[\alpha]_D$ -73 (*c* 0.41, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 4.94 (s, 1 H, H-1′), 4.8 (m, 1 H, H-1″), 4.64 (d, 1 H, $J_{1,2}$ 0.5 Hz, H-1), 4.36 (d, 1 H, $J_{2,3}$ 3.2 Hz, H-2′), 4.24 (d, 1 H, $J_{2,3}$ 2.8 Hz, H-2), 3.89-3.96 (m, 3 H, H-6a″, H-6a′, H-6a), 3.71-3.79 (m, 3 H, H-6b″, H-6b′, H-6b), 3.62-3.69 (m, 3 H, H-3′, H-3, H-4), 3.58 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.7 Hz, H-4′), 3.54 (s, 3 H, CH_3OC-1), 3.42-3.49 (m, 3 H, H-3″, H-4″, H-5″), 3.35-3.40 (m, 3 H, H-2″, H-5′, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 103.8 (C-1″, $^1J_{C1,H1}$ 166.7 Hz, β), 102.3 (C-1, $^1J_{C1,H1}$ 159.3 Hz, β), 101.4 (C-1′, $^1J_{C1,H1}$ 163.5 Hz, β), 78.1, 77.9 (C-2′, C-2), 77.2, 77.1 (C-2″/C-5′/C-5), 76.7(0), 76.6(8) (C-3″/C-4″/C-5″), 74.2 (C-2″/C-5′/C-5), 73.1, 72.9 (C-3′, C-3), 70.5 (C-3″/C-4″/C-5″), 68.2 (C-4), 67.8 (C-4′), 61.9, 61.6, 61.4 (C-6″, C-6′, C-6), 58.0 (CH_3OC-1); ESI HRMS Calc'd. for $C_{19}H_{34}O_{16}Na$ 541.1739. Found 541.1742.

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